DISEASES OF SEAWATER NETPEN-REARED SALMONID FISHES IN THE PACIFIC NORTHWEST

Dr. Michael L. Kent

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Abstract

Kent, M. L. 1992. Diseases of seawater netpen-reared salmonid fishes in the Pacific Northwest. Can. Spec. Publ. Fish. Aquat. Sci. 116: 000 p.

The important diseases and pathogens of seawater netpen-reared salmonid fishes in British Columbia, Canada and Washington, USA are described. Gross and microscopic characteristics of bacterial, viral, fungal, parasitic, algal, and idiopathic diseases are described. In addition, procedures for diagnosis, treatment, and control of each disease are outlined. The manual also includes a description of the general use of drugs in netpen aquaculture and regulations for their use in aquaculture in Canada. Methods for conducting a disease investigation at a netpen farm are also described.

Résumé

Kent, M. L. 1992. Diseases of seawater netpen-reared salmonid fishes in the Pacific Northwest. Can. Spec. Publ. Fish. Aquat. Sci. 116: 000 p.

On déorit les maladies et agents pathogènes importants dans l'élevage des salmonidés en parcs en filets dans de l'eau de mer en Colombie-Britannique (Canada) et aux États-Unis (É.-U.). On décrit les caractéristiques macroscopiques et microscopiques des maladies idiopathiques et des maladies causées par des bactéries, des virus, des champignons, des parasites et des algues. En outre, on souligne les méthodes utilisées dans le diagnostic, le traitement et la maîtrise de chacune de ces maladies. Le guide fait également mention des médicaments utilisés généralement en aquaculture en parc en filets et de la réglementation concernant leur utilisation en aquaculture au Canada. On décrit également les méthodes utilisées pour déterminer la nature des maladies qui peuvent se déclarer dans un établissement utilisant les parcs en filet.

Preface

Aquaculture is an important and expanding component of the fisheries industry in Canada. In British Columbia, the long coastline, temperate climate and clean environment make it especially suitable for salmonid husbandry. The landed value ($82.1 million) of salmon culture production in British Columbia in 1989 was estimated to be 35% of the B.C. commercial salmon fishery's 5 year average landed value ($236 million). Salmon production in B.C. is projected to increase by over 250% between 1989 and the year 2000.

Successful aquaculturists manage their operations in a manner that minimizes the impact of disease. This Manual, entitled "Diseases of Seawater Netpen-reared Salmonids in the Pacific Northwest", provides fish health specialists and culturists with a valuable reference on salmonid diseases in the region. It also represents an important milestone in meeting this Department's commitment to transferring information and technologies from our research laboratories to the aquaculture industry.

Finally, I am pleased to note that the Manual is a product of research conducted at the Pacific Biological Station, of interactions with other fish health workers in Canada and the USA and collaboration with salmon culturists. It is important that we maintain this spirit of cooperation between government agencies and the private sector, if we are to achieve the scientific advances that are so vital for growth in the aquaculture industry.

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February 4, 1992

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I thank the veterinarians and other fish health professionals in British Columbia and Washington State for providing material and information that was very helpful for preparing this manual. Most of the material from Washington State was obtained with the assistance of Drs. Ralph Elston and Lee Harrell. In British Columbia, Drs. Rob Armstrong, John Barrie, Jim Brackett, Don French, Brad Hicks, John King, Garret Newbound, Mark Shepard, and Carolyn Rymes provided assistance with obtaining material from netpens. Dr. Shepard was especially helpful with providing material and information on mxyobacterial stomatitis. I also thank Dr. Craig Stephen for preparing the line drawings in the text.

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Introduction

Culture of salmonid fishes in seawater netpens is rapidly expanding in the Pacific Northwest (British Columbia, Canada and Washington State, USA). Most research on diseases of salmonids has been directed towards those affecting fish during their freshwater phase of development. With the increase in seawater netpen aquaculture several new marine diseases of salmonid fishes have been recognized. Described here are important diseases and pathogens of salmonid fishes reared in seawater netpens in the Pacific Northwest. This text does not address diseases of importance in freshwater netpens, and henceforth "netpens" or "pen-reared" denotes seawater netpens. This manual is intended primarily for workers at netpen sites and field laboratories. Therefore, diagnostic characteristics of each disease that can be obtained by macroscopic observation, simple stains or wet mount preparations are emphasized. However, descriptions of histopathological changes are included to provide an understanding of the pathogenesis of each disease. It is expected that the manual will be used mostly by those with basic training in microbiology, parasitology, pathology, and fish health. However, I hope that aquaculturists in general will also make use of the manual, and a glossary is provided in the appendix to familiarize fish farmers with some of the technical terminology used in fish health. Preservative and media formulations, and scientific names of fishes referred to in the text are also listed in the appendix.

An abbreviated description of methods for conducting a disease investigation and a basic necropsy protocol are included. This manual is not intended to be a complete laboratory manual and more detailed descriptions of principles for diagnostics can be obtained in basic fish disease texts (e.g., Kabata 1985; Post 1987; Roberts 1989).

Information obtained in the field may be adequate for only presumptive diagnosis, and more in-depth laboratory investigations are often required for positive diagnosis of certain diseases. Therefore, methods for preservation and transport of specimens to a laboratory for further microbiological, histopathological, and chemical examinations are included.

Control of diseases is very important to the economic viability of netpen fish farms. It is hoped that this manual will help fish health practitioners and fish farmers identify diseases in pen-reared salmon, and that in doing so it will provide a sound basis for implementing appropriate control and treatment procedures.

Fish Health in Netpens - General Considerations

Rearing fish in netpens may be economically expedient because of the relatively low construction costs involved. Also, there is no need for pumping water. However, this form of aquaculture may allow for the exacerbation of certain diseases and presents some unique fish health problems. With uncontrolled exchange of water in netpens, there is the potential for introduction of pathogens, pollution, and noxious algae. Certain pathogens, such as external parasites, are often only a minor problem in hatcheries or ponds because, in most cases, they are quickly eliminated with externally applied therapeutic agents. In contrast, these pathogens can be devastating in netpens because it is extremely difficult to apply and maintain appropriate levels of bath-administered drugs in this situation. For example, external parasitic copepods are one of the most serious problems for pen-reared Atlantic salmon.

Fish in netpens will often feed on naturally occurring food organisms. These organisms may not normally be eaten by the fish and, when eaten, may result in unusual parasite infections (e.g., metacestodes of *Gilquina squali* - see page 44). In addition, netpen liver disease (page 62) may also be caused by feeding on naturally occurring netpen biota.

Although several causes of disease in pen-reared salmonids have been identified, there are few drugs available for their treatment. Therefore, most diseases are controlled by changing to husbandry practices that avoid infections or improve the overall health status of the fish. Many important diseases in netpens originate in fresh water (e.g., furunculosis and bacterial kidney disease). Often the best way to control these diseases is to identify fish in fresh water that have subclinical infections and to avoid their introduction into netpens.

Early detection of a disease is often difficult in netpens. Often when a disease problem is recognized by the fish farmer, the disease has advanced to a stage where, even with immediate action, high mortality is unavoidable. In netpens, it may be difficult to detect subtle external lesions on fish or behavioral changes indicative of the onset of disease. Furthermore, moribund or dead fish that accumulate at the bottom of nets may not be detected until they are collected by divers or until the nets are raised. Added to these

problems, a certain low-level of mortality is often considered acceptable by some fish farmers; these losses are classified as "natural mortality" and are often ignored. Fish in this category should, however, be examined because catastrophic disease outbreaks can usually only be avoided by early detection of a problem, and these fish sometimes represent the incipient stages of a serious disease. It is only by frequent and consistent examination of "morts" and by complete record keeping of mortalities in each stock that developing disease problems can be effectively circumvented or prevented.

When an organism is cultured in a new geographic area it is often subject to diseases that do not affect the indigenous species. The Atlantic salmon is a very desirable species for netpen culture. Because this fish is not native to the Pacific Northwest, it may lack innate resistance to certain indigenous disease agents, to which indigenous Pacific salmon are resistant. There are already examples of this phenomenon; Kent et al. (1988) described a toxic liver disease to which Atlantic salmon are more susceptible than Pacific salmon (see page 62), and Dr. Z. Kabata, Pacific Biological Station, Nanaimo, B.C. (pers. comm.) identified a copepod (*Haemobaphes* sp.) from pen-reared Atlantic salmon. This copepod has previously been observed only on non-salmonid fishes.

Often new diseases are observed when an organism is reared under new environmental conditions. The rearing of salmonids in netpens is a relatively recent form of aquaculture in the Pacific Northwest. It is, therefore, not a surprise that in the past 5 years at least six previously unreported diseases have been documented in pen-reared salmon in the Pacific Northwest, and it is very likely that more "new" diseases will be observed. Some of these diseases may be caused by previously unknown organisms (e.g., the rosette agent). Whereas others are caused by organisms that are known, but which appear to be less pathogenic in freshwater or in wild salmon (e.g., *Loma salmonae*). The high likelihood of encountering a new disease in the netpen situation is a problem for the diagnostician because he/she cannot always rely on published references or past experience for making quick disease diagnoses.

DISEASE TREATMENT IN NETPEN AQUACULTURE

By J. Brackett and M. L. Kent

Treatment of fish reared in netpens can be accomplished in a number of different ways, all of which have some associated benefits and disadvantages. All methods of treatment have some similar prerequisites. An accurate diagnosis of the condition to be treated and the extent of its likely impact on the fish in the netpen is essential. Sampling of fish should be done so as to determine the reasons for the morbidity or mortality. This is not always an easy task in netpen situations. In addition, thorough necropsy examinations are needed to accurately diagnose the problem. For example, many cases of bacterial kidney disease (BKD) in Atlantic salmon are missed if the brain is not examined and sampled. An accurate estimate of the number and biomass of fish in the netpen is needed so that the amount of medication needed for the treatment can be calculated. This requires reliable records of average fish weight and losses. During treatment, fish should be handled carefully to prevent exacerbation of the disease or initiation of additional problems. Handling should be done so that stress and physical trauma are minimized and so that proper oxygen levels are maintained throughout the treatment.

Medicated Feed

Delivery of drugs through medicated feed is the most common method of treating fish in netpens. It is the least labor-intensive method and may provide the least stress to the treated fish. Antibiotics are the drugs most commonly administered in the feed. Drugs are added to the feed during milling at the end of pellet manufacturing or they are applied to the pellets at the farm using oil or gelatin as a carrier. At the farm, the drug is mixed into the feed in a wheelbarrow or cement mixer, depending on the type and amount of drug required. Commonly used antibiotics are available from feed manufacturers quickly in the event of a disease outbreak.

There are, however, several disadvantages associated with administering drugs in feed. Diseased fish often are reluctant to feed and the amount of feed consumed may vary substantially between fish. Some drugs also reduce the palatability of feed, further adding to the treatment problem. If feed is not consumed efficiently, waste of drugs and environmental problems may result. Some drugs (e.g., oxytetracycline) also interact with components in the feed so that their availability and efficacy are altered. In many cases the drug is not supplied in a pure form. This fact must be taken into account if the correct dosage of the prue drug is to be administered.

Injection of Drugs

This method is practical only when the number of fish to be treated is relatively small and the value of the individual fish is high, such as with brood stock. For example, bacterial kidney disease is controlled in brood stock by injections of erythromycin. Injections may be given intramuscularly, intraperitoneally or by the dorsal sinus. Although the drug is delivered accurately and efficiently by injection, the labor and handling involved are higher than for other treatment methods. Similarly, the stress associated with handling in the fish is likely to be high and should be reduced by the use of anesthesia.

Bath and Immersion

In contrast to freshwater hatchery situations, bath or immersion treatments are applied only with difficulty in netpen farming. Removing the fish from netpens for immersion in solutions of compounds is a labor intensive procedure. Instead, bath treatments are usually applied directly to pens. Before the treatment is administered, the pen is enclosed with a plastic sheet to prevent water movement out of the pen and to maintain the appropriate concentration of the drug. Adequate oxygenation and gentle handling of the fish must be ensured in order to reduce stress and trauma during treatment.

Canadian Regulations for the Use of Therapeutic Agents in Aquaculture

In Canada, compounds used to treat or prevent fish diseases or to control pathogens are grouped into three major categories for regulatory purposes. The first category includes all compounds classified as drugs. The Food and Drugs Act defines a drug as

> "any substance or mixture of substances for use in (a) the diagnosis, treatment, mitigation or prevention of a disease, disorder, abnormal physical state, or the symptoms thereof, in man or animal or, (b) restoring, correcting or modifying organic functions in man or animal".

Drugs are regulated by the Bureau of Veterinary Drugs, Health and Welfare Canada. The second and third categories include biologics (vaccines and immunoglobulins) and pesticides, which are regulated by Agriculture Canada. The province of British Columbia has additional regulations governing record-keeping, reporting and drug withdrawal time (the period between cessation of treatment and fish harvest).

1. DRUGS

There are only three drugs licensed for use in fish in Canada: Terramycin-Aqua (oxytetracycline, Pfizer), Romet-30 (sulfadimethoxine and ormetoprim, Hoffman-La Roche) and Marinil (metomidate, Wildlife). Although not licensed for use in fish, any drug licensed in Canada for use with any other animal species or humans may be used in fish if prescribed by a veterinarian. In this situation, the veterinarian is responsible for toxic side effects or tissue residue problems. In order to provide a prescription allowing a producer to use a drug or medicated feed, a veterinarian requires information including: 1) a knowledge of the producer's operation and fish; 2) the producer's name, address, and telephone number; 3) the disease or condition to be treated and a record of how the condition was diagnosed; 4) details of the fish species, numbers, ages, and sizes; 5) the form and dose of the drug to be used; 6) the method of administration of the drug; 7) the type of the feed and feeding rate; and 8) the water temperature.

1.1 Non-prescription Drugs Licensed for Fish.

Terramycin-Aqua (oxytetracycline) is the only drug licensed for use in salmon and trout that does not require a prescription from a veterinarian. This drug may be used to medicate feed without a prescription for the treatment of ulcer disease caused by *Haemophilus piscium*, furunculosis caused by *Aeromonas salmonicida*, columnaris disease caused by *Chondrococcus (Flexibacter) columnaris*, coldwater disease caused by *Cytophaga psychrophila*, and enteric redmouth disease caused by *Yersinia ruckeri* in salmon and trout. It may be used to medicate feed to deliver oxytetracycline at 75 mg drug/kg of fish/day for 10 days. If any other dose is to be used or any other disease (e.g., vibriosis) is to be treated, a veterinary prescription is required. Following treatment, fish may not be harvested or released for at least 40 days when the water temperature is 10°C or higher, or 80 days when the water temperature is below 10°C.

1.2 Prescription Drugs Licensed For Fish

Sulfadimethoxine and *Ormetoprim (Romet-30).* Romet-30 is licensed for prescription use for the treatment of furunculosis in salmonids caused by *Aeromonas salmonicida*. It is administered in the feed and the dosage is 50 mg of active ingredient/kg fish/day for five days. After treatment fish must not be harvested for at least 42 days if the water temperature is 10°C or higher. Romet-30 is not licensed for use when the water temperature is less than 10°C, but a veterinarian may prescribe its use at lower temperatures. The prescribed withdrawal time in this case may be longer than 42 days. Romet-30 is also often prescribed for treatment of vibriosis or for myxobacterial stomatitis (mouth rot) at similar doses as for furunculosis treatment.

Oxytetracycline. This drug is often used for medicated feed treatments other than the non-prescription uses for Terramycin-Aqua. Therefore, a prescription from a veterinarian is required for these additional applications. Common uses include treatment of furunculosis or vibriosis at 100 mg drug/kg fish/day for 10 days, and treatment of bacterial kidney disease at 100 mg drug/kg fish/day for 21 days.

Many oxytetracycline products are licensed in Canada for use in non-fish species (e.g., Oxysol, Sanofi). These products may be used in fish by prescription. The prescribed withdrawal time for these forms of the drug may be different from that for Terramycin-Aqua.

Oxytetracycline is also given by dorsal sinus injection to brood stock for prevention and treatment of furunculosis and BKD. Long-acting preparations of oxytetracycline at 200 mg/ml are used at a dose of 20 mg/kg of fish in one injection. This use of the drug is not licensed, so a veterinary prescription is required. Because injection of oxytetracycline may result in high drug levels in treated fish for a long time, an extended withdrawal period is usually prescribed by the attending veterinarian.

Marinil. Marinil (Wildlife, Syndel) is used for tranquillizing and anaesthetizing fish for weight sampling, injections, sorting, transporting, etc. Marinil is licensed for use in non-salmonid species, but may be used in salmon and trout by veterinary prescription. Doses in a bath of about 0.25 ppm are used for tranquillisation and 1 to 5 ppm are used for anaesthetization. A withdrawal time of about 40 days is commonly prescribed when water temperatures are 10°C or higher.

1.3 Prescription Drugs Licensed for Other Animals

Erythromycin. This drug is commonly used in brood stock females for prevention of egg infections with *Renibacterium salmoninarum*, the causative agent of BKD. It is given by injection into the dorsal sinus at 20 mg drug/kg fish. An injectable product licensed for non-fish species is used (Gallimycin, Sanofi), with a concentration of erythromycin of 200 mg/ml. Pacific salmon brood stock treated with erythromycin are not to be used for food, whereas treated Atlantic salmon brood stock require a long period of reconditioning before harvest.

Erythromycin feed additives (e.g., Gallimycin, Sanofi) are sometimes used for BKD treatment via the feed, but the cost of treatment is high relative to oxytetracycline. Recent trials have shown no cost-benefit to using erythromycin in place of oxytetracycline for BKD treatment in netpens. The dose used is 100 mg erythromycin/kg fish/day for 10 days or more. The prescribed withdrawal time is often several months.

Ivermectin. Ivermectin (Ivomec, MSD Agvet) is used for the treatment of sea lice. The product licensed for drenching sheep is often used. It is added to feed at the farm to yield levels such as 0.025 or 0.05 mg drug/kg fish for 5 or more treatments at 4 to 7 day intervals. The dose may have to be adjusted as the margin between the effective dose and the dose toxic to fish can be quite small depending on water temperature, condition of the fish, and the presence of other diseases. The prescribed withdrawal time is usually 6 months or more.

Nitrofurazone, Sulfamerazine and Similar Drugs. Sulfa drugs and nitrofurazone and related drugs are no longer commonly used due to the rapid development of strains of bacteria resistant to these drugs and concerns with the potential for unacceptable drug residues in treated fish.

1.4 Drugs Not Licensed in Canada

Drugs of value for treatment of fish, but not licensed for use in Canada in fish, higher vertebrates or humans are sometimes available by special approval from the Bureau of Veterinary Drugs. These drugs require either an Emergency Drug Release (EDR) or an Experimental Studies Certificate (ESC) from the Bureau of Veterinary Drugs before a veterinarian can prescribe their use. The veterinarian is required to provide all of the information normally required for a prescription, and in addition a complete protocol for the use of the drug. Following each use of the drug, the veterinarian must write a follow-up report, describing the results of the treatment and any adverse reactions. Approval for an EDR or ESC may take from 1 or 2 days to 2 weeks or more.

Tricaine Methanesulphonate (TMS, MS-222). This compound is used as an anesthetic for salmonids. Although it has been an essential drug in fish culture in Canada for decades and is licensed in the U.S., it is not yet licensed in Canada. It is commonly used at about 40 ppm in a bath. The prescribed withdrawal time before harvest is 5 days following treatment.

Benzocaine. Benzocaine is another anesthetic used in a similar manner as TMS. It has the disadvantage of being relatively insoluble in water, requiring dissolution in a stock solution of solvent before use.

Oxolinic Acid, Flumequin. These two antibiotics belong to the quinolone group of drugs. They have been used very effectively in the past for *Aeromonas salmonicida* infections when the bacteria are resistant to oxytetracycline and Romet-30 (an increasingly common finding). The Bureau of Veterinary Drugs has recently established a policy of refusing to permit the use of all quinolone drugs under EDR or ESC. This policy renders these drugs unavailable for use until extensive studies are conducted in Canada. It is unlikely that these studies will ever be conducted.

Other Drugs. Many other drugs licensed for use in non-fish species are currently used infrequently or have the potential for use in salmon and trout. These include: Virginiamycin (SmithKline Beacham) for BKD, Fumagillin (Sanofi) for protozoan infections, Albendazole (SmithKline Beacham) for *Philonema* sp. nematode infections, and Praziquantel for tapeworm infections.

2. BIOLOGICS

The manufacturing, testing and sale of biologics is regulated by Agriculture Canada. Vaccine sales are made directly to users. Veterinary prescriptions are generally not required for use of vaccines.

Vaccines. Several vaccines are licensed by Agriculture Canada for use in salmonids. Only *Vibrio anguillarum* bacterins are commonly used

in netpen culture. Juvenile salmon are usually vaccinated for vibriosis in fresh water about 4 wk before introduction to seawater. In addition, smolts are commonly revaccinated by immersion during their first month in netpens. This provides an increase in protection from vibriosis over that produced solely by vaccination in the hatchery.

Immunoglobulins. Immunoglobulins produced in mammals and purified for injection to fish are licensed for the treatment and prevention of *Aeromonas salmonicida* infections in salmonids. These are given by intraperitoneal injection (0.4 ml/kg fish) to brood stock before spawning, as well as to pre-smolts.

Autogenous Vaccines. These vaccines are produced from a pathogen cultured from fish on a farm and are intended for use on the same farm. Agriculture Canada allows a vaccine manufacturer to produce these vaccines for use only on the farm from which the pathogen was obtained. This allows the producer to use a vaccine containing the specific strain of pathogen found on the farm.

3. PESTICIDES AND DISINIFECTANTS

Pesticides are compounds used for the control of external parasites. Disinfectants are compounds used on inanimate surfaces to control pathogens. Both of these classes of compounds are regulated by Agriculture Canada.

There are currently no pesticides licensed for use with fish in Canada. The requirements for the licensing of such products are stringent, requiring many years of testing and great expense. Strong interest in the use of pesticides such as organophosphates (e.g., Nuvan, Ceiba-Geigy), pyrethrins and pyrethroids for treatment of sea lice, has led to efforts by producers to provide support for the licensing of products. There is no provision for making pesticides available under a system such as an EDR, and no provision for use by veterinary prescription under Agriculture Canada regulations. Thus, these products are essentially unavailable for use in netpens.

Most disinfectants used on netpens are approved for common uses, such as for foot baths and disinfection of nets. These include iodine disinfectants, bleach, quaternary ammonium compounds and formalin. Although formalin is licensed in the U.S. for treatment of fish diseases, it and other disinfectants are not specifically licensed for fish disease treatment in Canada. It is often unclear as to whether a compound is being used as a drug, requiring licensing for that use, or as a disinfectant. The agency responsible for controlling the use of the compound is, therefore, sometimes uncertain.

All drug uses, doses, and withdrawal times given in this chapter are for information only, and are not to be considered as recommendations. Because regulations and uses change often, consult your veterinarian before the use of drugs.

DISEASE INVESTIGATION IN NETPENS

The mere presence of a pathogen in a sick fish does not necessarily mean that it is the cause of the disease. It is important, therefore, to conduct a thorough investigation, beyond just detecting the presence or absence of known pathogens, to determine the cause of the disease. Histological examinations, in addition to gross necropsies and bacteriological cultures, are often required to determine the association of the observed pathogens in the disease being investigated. Furthermore, because fish in netpens often have mixed infections, it is often necessary to examine several fish from the affected population to differentiate primary pathogens, opportunistic pathogens, and secondary causes of the disease.

Diagnosis of a disease begins at the farm, and some of the most crucial information can be collected by the farmer by thorough record keeping and careful observations of affected fish. The following information is important for disease diagnosis and implementing control strategies.

History. The disease and medication history of individual stocks are helpful for early recognition of an emerging disease problem. During a disease outbreak the following information should be obtained: time of seawater entry, previous disease problems in the affected stock in fresh water and in the netpen, diet, medication history, and mortality rate.

Water Quality. Suboptimal water conditions often exacerbate diseases and, because of lack of control of water movement in netpens, water quality problems may be unavoidable. The following are water quality parameters of particular concern for netpens and should be considered in a disease investigation: dissolved oxygen, temperature, salinity and phytoplankton density.

Behavior and Clinical Signs. Fish behavior may be useful for indication of an emerging disease and some behavior changes are useful for presumptive diagnoses. For example, if fish are flashing (rubbing on nets), this may indicate that they are infected with external parasites. Other indications of disease include: cessation of feeding, lethargy, and abnormal position in the netpen (e.g., at surface or at the bottom). Abnormal respiratory pattern may indicate gill damage, and whirling or spiralling swimming often indicates neurological damage.

Necropsy Protocol

Selecting fish for Necropsy. Examinations should be conducted on diseased fish that are collected while still alive (moribund). It is important to determine the primary cause of mortality and to differentiate this from secondary or opportunistic pathogens that may have taken advantage of already diseased fish. To accomplish this, several affected fish should be examined whenever possible. In addition, the sample should include apparently normal, asymptomatic fish so that early pathological changes and the underlying cause of morbidity can be determined. Dead fish may be suitable for some parasitological examinations and for observing obvious macroscopic pathological changes. However, bacteriological examinations conducted on dead fish can yield misleading results, and many histological changes are obliterated by post-mortem autolysis.

External Examination. Note surface abnormalities (e.g., frayed fins, cloudy eyes, ulcers, skin discolorations, parasites, and tumors). Prepare wet mounts of the skin mucus and a few scales by scrapping the surface of the fish with a coverslip and placing the coverslip on a glass slide. Some sea water may be added to the preparation so that the area between the slide and the coverslip is completely filled with liquid. Examine the wet mount with a compound microscope, starting with low power. Reducing the light and lowering the condenser will produce higher contrast, making microscopic parasites and other pathogens more visible.

Gills. Remove the operculum. Note color of gills (pale gills may indicate anemia). Check for parasites, excessive mucus, and for hemorrhages. A dissecting microscope is useful for detecting larger parasites. Prepare a wet mount of a few filaments and examine for small parasites, fungi, and bacteria using a compound microscope. Wet mounts of gills are prepared by removing a few filaments with scissors, placing the filaments in a large drop of sea water on a glass slide, and overlaying with a coverslip.

Internal Examination. Open the visceral cavity. Note if ascites, hemorrhages or other abnormalities are present. Expose the kidney by removing the swimbladder and note any kidney abnormalities. Many diseases cause enlargement or discoloration of the kidney. Examine the brain

and heart for any abnormalities. Remove and open the intestinal tract by cutting length-wise and examine for parasites with a dissecting scope or magnifying glass. Dissection of the gastrointestinal tract in 0.9% saline is helpful for finding helminth parasites. Examine squash preparations of organs with a compound microscope to detect encysted parasites, fungi or granulomas. Squash preparations are made by removing a small piece of tissue, and gently squashing it between a slide and coverslip so that a thin preparation suitable for examination with a compound microscope or dissecting microscope is made.

Imprints. Leishmans's Giemsa (see Appendix III) or Dif-Quik (Harleco) stained imprints of kidney, spleen or other affected organs are often useful for detection of protozoa and bacteria. Remove a piece of tissue (approximately 0.5 X 0.5 cm), blot on clean paper towel to remove most of the blood, and lightly touch the cut surface of the tissue on a clean glass slide. Several imprints from the same piece of tissue can be made on one slide. Air dry the preparation for approximately 1/2 h and fix the slide for 5-10 min in absolute methanol. The slide can then be stained or shipped to a diagnostic laboratory.

Bacteriology. Some bacterial diseases, such as bacterial kidney disease, can be identified by Gram stain. However, the diagnosis of most bacterial diseases requires isolation and identification of the bacteria in culture. In this case, live fish should be delivered to the laboratory, but this may not practical in some situations. For this reason, methods for obtaining initial cultures in the field are outlined below. The bacterial cultures can then be sent to a laboratory for complete identification.

Use only freshly killed fish showing clinical signs. Dead fish from the pens are essentially worthless. Disinfect the surface of the fish with 70 % ethanol, flame-sterilize a scalpel, open the visceral cavity making sure not to cut into the gastrointestinal tract. Push aside the swimbladder with flame-sterilized forceps and insert a sterile swab or loop into the kidney. Streak the specimen on Tryptic Soy Agar and Marine Agar (Difco) bacteriological plates, seal the plates, keep at a temperature of 15-25 °C, and send the plates to a microbiology laboratory for further diagnosis.

Gram-stained preparations may reveal bacteria when they are numerous in infected tissue. Smear suspect tissues thinly on a glass slide, air dry and fix the slide by gently heating the slide over an open flame for 3-5 seconds. Gram stain kits are available from scientific supply houses and include instructions for their use.

Virology. As with bacterial diseases, isolation of viruses in culture may be required to diagnose a viral disease, and culture is best conducted on specimens collected from freshly killed fish. If this is not practical, the fish should be refrigerated for no longer than 24 before examination. As a last resort, fish for virus examination can be frozen. The specimens are then transported to a qualified fish virology laboratory.

Preservation and Transport of Specimens

It is not always possible to obtain live or fresh specimens. If only dead fish can be provided for laboratory examination, they should be refrigerated and examined within 24 h. If fish cannot be delivered to a laboratory within 24 h, the fish should be preserved by freezing and/or fixation in tissue preservatives. We have found Davidson's solution (see Appendix III) to be the best all around fixative for fish tissues for histological examination. Each method of preservation has certain advantages and disadvantages, as indicated in Table 1.

Preservation and Transport of Tissues in Fixative. It is critical to fix tissues for histology as soon as possible after fish are killed to avoid post-mortem changes. If possible, do not use dead fish from the netpens because significant autolytic changes may occur in 15-20 min after death. Fix tissues in Davidson's fixative for histology. Place small pieces of each organ in the fixative at approximately 1:20 (v/v) tissue to fixative. For shipment of fixed tissues, drain off excessive fluids, wrap the tissues in a fixative-soaked paper towels, and seal in a leak-proof container.

Transport of Frozen Fish. Freeze fish in a plastic bag and ship in an insulated container with ice packs. Freezing causes significant artifacts for histologic examination. Therefore, if histopathologic evaluation is anticipated, fish should not be frozen.

Transport of Refrigerated Fish. Place fish in a plastic bag and surround the bag with ice or ice packs. Ship in a leak-proof, insulated container.

Table 1.

Preservation methods of fish tissues and their uses in fish disease diagnostic examinations. +++ = optimal; ++ = satisfactory in most cases; + = suboptimal, can be used if no other tissue available; 0 = useless.

	Fresh	Refrigerated	Frozen	Preserved*
Parasitology	+++	++	++	+
Bacteriology	+++	+	+	0
Virology	+++	++	+	0
Toxicology (chemical analysis)	+++	++	+++	0
Histology	+++	++	+	+++

*preserved in formalin-based fixative (e.g., Davidson's solution).

BACTERIAL DISEASES

By T. P. T. Evelyn and M. L. Kent

There are five recognized bacterial diseases of pen-reared salmon in the Pacific Northwest: bacterial kidney disease (BKD), caused by *Renibacterium salmoninarum*; vibriosis, caused by *Vibrio anguillarum* and *V. ordalii*; furunculosis, caused by *Aeromonas salmonicida*; myxobacteriosis, caused by *Cytophaga-Flexibacter* spp.; and a systemic disease caused by a rickettsia, *Piscirickettsia salmonis*. All salmon species reared in netpens are susceptible to these bacterial diseases, but some diseases are more problematic in certain species than in others. For example, chinook, coho, and sockeye salmon appear to be more susceptible to BKD than Atlantic salmon, whereas furunculosis and myxobacteriosis are more of a problem in Atlantic salmon than in Pacific salmon species.

Bacterial diseases probably cause more mortality in pen-reared salmon than diseases due to other infectious agents. However, unlike most other diseases of fish, there are commercially-available drugs that may be efficacious for treating bacterial diseases. In addition, BKD and furunculosis can be managed to a certain extent by screening fish and avoiding infections, and efficacious vaccines are available for the prevention of vibriosis.

Bacterial Kidney Disease

Bacterial kidney disease is probably the most significant cause of mortality in pen-reared chinook and coho, but Atlantic salmon are also susceptible to the disease, although to a lesser degree (Brackett et al. 1991). In addition, sockeye, pink, and chum salmon are extremely susceptible to BKD (Brett et al. 1978; Evelyn 1988a), but these species have thus far been reared in netpens mostly on an experimental basis. The causative agent, *Renibacterium salmoninarum*, is a non-motile Gram-positive bacterium that infects inflammatory cells, primarily macrophages, and produces a systemic infection. It induces severe, chronic inflammation in the kidney, other visceral organs, the eye, brain, and to a lesser extent the muscle.

All ages of salmon are susceptible to BKD. The bacterium is transmitted vertically through the egg (Bullock et al. 1978; Evelyn et al. 1986). Clinical disease may occur in fresh water. In addition, fish can carry the infection with them to sea water, and may serve as a source of infection for their cohorts. In netpens, these fish may show signs of clinical disease shortly after introduction to seawater. However, most epizootics start in the first winter following the introduction of the fish to netpens, and peak in the following spring. These epizootics are probably due to horizontal transmission of the pathogen from heavily infected older fish, already on the farm. The seasonality of BKD appears to be diminishing, and outbreaks have been observed throughout the year.

CLINICAL SIGNS AND GROSS PATHOLOGY. Fish with BKD can exhibit a variety of macroscopic changes, often depending on whether the disease is present in acute or chronic form, or on the tissues affected. Affected fish may be dark, lethargic, and exhibit a swollen abdomen due to ascites. Some fish may exhibit exophthalmus and other eye lesions, and multiple blood-filled blisters may be present on the skin. The gills are usually pale due to anemia (Fig. 8, pg. 25). Internal examination usually reveals multifocal, greyish-white nodules in the kidney; these lesions may also occur in the spleen and liver (Fig. 8). Some fish may exhibit only a few, very large granulomas, whereas others show miliary lesions through out the viscera. Formation of a white, diffuse membranous layer (pseudocapsule) over the spleen and heart is also a frequent finding (Fig. 8). In acute cases, where the lesions are very diffuse, the granulomas may not be visible to the naked eye. The kidney and spleen are usually enlarged, and serosanguinous or cloudy fluid often accumulates in the visceral cavity. Hemorrhages are often observed in the liver, intestine, pyloric fat, and muscle. Large, focal, cystic cavities may also occur in the skeletal muscle. In some cases, the meninges are the only tissues in the fish that are severely affected. These fish exhibit spiralling or whirling swimming behavior, and usually show no macroscopic changes in the viscera.

MICROSCOPY. Gram-stained smears of infected organs from fish with clinical BKD exhibit numerous, small (0.5 X 1 µm) Gram-positive bacilli, many of which occur in aggregates within phagocytic cells (Fig 10). Histologically, BKD is best described as a bacteremia, characterized by systemic, diffuse chronic inflammation (Wolke 1975; Bruno 1986). Multifocal, coalescing granulomas are found in all affected tissues (Fig. 1). In Pacific salmon, the granulomas are diffuse with poorly defined borders, whereas the granulomas in Atlantic salmon are more

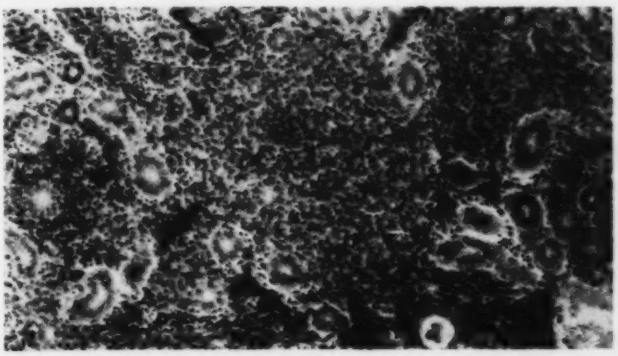


Figure 1. Tissue section of kidney from a chinook salmon with BKD. Note the granuloma in the kidney interstitium demarked by arrows. H & E.

encapsulated and contain numerous epithelioid cells. Caseation may occur in the centers of older granulomas, giving rise to cystic lesions. Tissue sections stained with Periodic acid-Schiff (PAS) reagent will readily reveal the organism within phagocytic cells in the granulomas (Fig. 10).

DIAGNOSIS. Presumptive diagnosis is achieved by observing the characteristic granulomatous lesions in affected fish. However, other pathogens may cause granulomatous lesions (e.g., *Ichthyophonus hoferi*, encysted parasites). In addition, not all fish with BKD exhibit macroscopically visible granulomas. Therefore, confirmatory diagnosis of the disease is based on the detection of the bacterium in either Gram-stained smears or PAS-stained tissue sections along with the characteristic histological changes.

A number of diagnostic tests have been developed to detect the bacterium in subclinical infections. Serological methods such as the indirect and direct fluorescent antibody tests (Bullock and Stuckey 1975a; Bullock et al. 1980) are used to screen smolts for the infection before their transfer to sea water, and the coelomic fluid of ripe females is examined using these methods to avoid infected eggs. Elliot and Barila (1987) described a membrane filtration technique to enhance the detection of the bacterium from coelomic fluid. This test is extremely sensitive, and is about 100 times less likely to overlook the presence of the pathogen in the coelomic fluid than the fluorescent antibody tests mentioned above (Lee and Evelyn, unpubl. data).

An ELISA test to detect *R. salmoninarum* antigens in fish tissues has also been developed by Pascho and Mulcahy (1987). This test is very sensitive and is particularly well adapted for screening large numbers of fish for the presence of the pathogen. The test shows great promise as a tool for selecting brood stock or smolts that are free of *R. salmoninarum*, and may provide the basis for avoiding infections with the bacterium (Pascho et al. 1991)

Renibacterium salmoniarum can be cultured on enriched media, such as KDM-2 (Evelyn 1977) or charcoal agar (Daly and Stevenson 1985). Recently Evelyn et al. (1989; 1990) developed special culture techniques for enhancing the growth of this fastidious and slow-growing bacterium, thus greatly increasing the speed and sensitivity with which the bacterium can be cultured. Notwithstanding this, culture cannot be recommended as a routine method for detecting the infection. It is a lengthy procedure and overgrowth of the culture plates with fast-growing contaminating bacteria often occurs even when antibiotics for suppressing these contaminants are included in the culture medium (Austin et al. 1983).

CONTROL AND TREATMENT: Elliot et al. (1989) recently reviewed control strategies for BKD. Vaccines effective against BKD are lacking and the disease is difficult to treat with antibiotics.

Oral treatment with erythromycin phosphate is efficacious for BKD (Groman and Klontz 1983; Austin 1985), but does not eliminate the infection from all fish (Wolf and Dunbar 1959; Austin 1985). In addition, fish may reject feed medicated with erythromycin, which may reduce the effectiveness of the drug (Schreck and Moffitt 1987). Furthermore, this drug is too expensive to use for production fish in netpens, and it is not licensed for use in fishes. Because of cost-considerations, oral treatment with oxytetracycline has routinely been used to treat BKD in netpens throughout the Pacific Northwest. However, reports on well-controlled studies to evaluate the efficacy of oxytetracyline for controlling BKD in netpens appear to be lacking.

Although impractical in production situations in netpens, injection of erythromycin is useful for controlling BKD in brood stock and for preventing egg-mediated parent-to-progeny (vertical) transmission of the bacterium (Lee and Evelyn 1991). Fish are injected medially between the epaxial muscles, just anterior to the dorsal fin, with 20 mg erythromycin/kg fish 9 - 57 days before spawning. Eggs from females treated in this manner contain concentrations of antibiotic sufficient to kill the bacterium in eggs. As an additional precaution, eggs from treated broodstock should be surface-disinfected with an

iodophore (100 ppm iodine for 15 min) to eliminate the pathogen on the egg surface.

To summarize, the best way to control BKD is to avoid the infection. Although most stocks of chinook in B.C. are probably infected with *R. salmoninarum*, avoidance of the pathogen should still be possible. Ideally, eggs from infected females should not be used. In addition, because no diagnostic test will detect all infected brood stock, the females serving as sources of eggs should be injected with erythromycin as just described shortly before spawing. Resulting fry should be reared in fresh water that is not contaminated by infected fish, and, if practical, only smolts that are apparently free of the pathogen should be transferred to netpens.

Avoiding horizontal transmission in the netpens is also very important. Bacterial kidney disease is transmissible in sea water (Evelyn 1988a; Murray et al. 1992), and many smolts probably contract the infection from heavily infected fish of the previous year class that are maintained on the same site. Therefore, it is highly advisable that only one age group of fish be held at each site.

Vibriosis

Vibriosis is a systemic disease that affects many marine fishes and invertebrates (Anderson and Conroy 1970; Colwell and Grimes 1984; Egidius 1987). Fierichs and Roberts (1989) considered vibriosis to be the most significant disease of wild and cultured marine and brackish water fishes. In salmonids, the disease is caused by *Vibrio anguillarum*, *V. ordalii*, and *V. salmonicida*. The former two bacteria cause disease in pen-reared salmonids in the Pacific Northwest (Evelyn 1971; Harrell et al. 1976; Novotny 1978; Schiewe et al. 1981). To date, however, *V. salmonicida* has not been detected in the Pacific Northwest. *Vibrio salmonicida* causes Hitra disease (also know as coldwater disease) in Atlantic salmon in Europe (Egidius et al. 1981, 1986; Bruno et al. 1986) and has been isolated from pen-reared Atlantic salmon in the Maritimes.

Vibrio anguillarum accounts for the vast majority (over 95%) of the outbreaks of vibriosis occurring in the Pacific Northwest. In contrast, *V. ordalii* causes only infrequent and sporadic problems. *Vibrio anguillarum* has been intensively studied for many years because it is a major cause of vibriosis in several fish species worldwide. Unlike *V. ordalii*, strains which form a homogenous group, *V. anguillarum* strains show much heterogeneity. This heterogeneity is both phenotypic (Tajima et al. 1985) and serotypic (Kitao et al. 1984; Tajima et al. 1985; Sorenson and Larsen 1986). Fortunately, the strains of *V. anguillarum* that cause vibriosis in the Pacific Northwest represent only a single serotype (based on the "O" antigens present). This greatly facilitates serological identification of the organism in disease outbreaks and it also simplifies the formulation of vibriosis vaccines for controlling vibriosis in the Pacific Northwest.

Vibriosis can cause high, acute mortality in unvaccinated smolts, and mortalities as high as 90% have been reported (Cisar and Fryer 1969). Although all *Vibrio* species, except *V. cholera*, are considered to be marine bacteria, outbreaks of vibriosis occasionally occur in freshwater-reared salmon . The source of the bacterium in freshwater situations is not always clear, but in some instances it was probably a diet that was formulated with marine products (reviewed by Evelyn 1988b). Vibriosis outbreaks are favored by temperatures between about 15 and 21 °C, and most outbreaks occur in smolts during their first summer in sea water. Vibriosis in the Pacific Northwest is mainly a problem of Pacific salmon (e.g., chinook and coho), but Atlantic salmon are also susceptible.

CLINICAL SIGNS AND GROSS PATHOLOGY. Mortality caused by vibriosis may be very severe and rapid, and moribund fish in such cases may exhibit no gross pathological changes other than darkening and lethargy. As typical of septicemias caused by Gram-negative bacteria, fish with vibriosis may exhibit erythema at the base of the fins, petechiae in the skin, and exophthalmus (probably due to kidney failure). Fish may also exhibit skin ulcers and frayed fins. Internally, congestion and petechiae are usually evident in the visceral organs, particularly in the gut and liver. Large, multiple coalesing hematomas in the liver are often seen in vibriosis (Fig. 8, pg. 25). These lesions appear to be unique to vibriosis, as we have not seen them in pen-reared salmon with other diseases. Affected fish also exhibit pallor of the gills (due to anemia) and enlargement of the spleen and kidney.

MICROSCOPY. Histological changes are consistent with septicemias caused by Gram-negative bacteria (Ransom et al. 1984). With *V. anguillarum*, the bacterium occurs as single cells that are distributed throughout the vasculature. Necrosis and edema are associated with the infection in well-vascularized organs such as the liver, kidney, and spleen. Increased deposition of hemosiderin in the spleen and kidneys may be

observed. Fish may also exhibit severe cardiomyopathy. *Vibrio ordalii* infections, on the other hand, are characterized by focal lesions, and large colonies of the bacterium may be observed in the affected tissues (Ransom et al. 1984).

DIAGNOSIS. Presumptive diagnosis is possible by macroscopic examination if the characteristic hematomas in the liver are present (Fig. 8). The other gross and clinical changes are not specific to vibriosis and are associated with a number of bacterial or viral systemic diseases. Confirmatory diagnosis is based on culture and identification of the causative organism from the kidney of suspect fish. Both *V. anguillarum* and *V. ordalii* are easily cultured on Tryptic Soy Agar with 1.5 % NaCl or on Marine Agar (Difco) at room temperature. Bacterial colonies are round, raised, and off-white in color. *Vibrio ordalii* grows more slowly than *V. anguillarum* and forms smaller colonies. Pure cultures of the suspect bacteria can be distinguished from one another using biochemical tests (Schiewe et al. 1981; Tajima et al. 1985; Scalati and Kusuda 1986). API-20E test strips (Analytab Co., Analytab Products, Plainview, NY, USA) can be used for rapid and easy identification of marine vibrios from fish (Kent 1982; Grisez et al. 1991). Using the API-20E test, *Vibrio anguillarum* is distinguished from other Gram-negative bacteria causing disease in fish by the following criteria: the bacterium yields positive oxidase, Voges-Proskauer, and gelatinase reactions; ferments sucrose, sorbitol, and arabinose; does not grow at 40°C; and does not produce gas. *Vibrio ordalii*, in contrast, is Voges-Proskauer negative and does not ferment sorbitol or arabinose. Both bacteria are sensitive to the vibriostatic compound O/129 (2:4 diamino 6:7 diisopropyl pteridine). These bacteria can also be identified serologically using slide agglutination tests. Rabbit antisera required for the tests are available commercially (Microtek R & D, Ltd., Victoria, B.C.). Interestingly, fluorescent antibody tests applied directly to vibrio-infected tissues cannot be used for rapid diagnosis of the disease (Evelyn, T. P. T., unpubl. data). Apparently salmonid tissues (including tissues from naive fish) contain substances that block receptor sites on the vibrios that would normally react with the vibrio-specific antibodies in the diagnostic antisera.

CONTROL AND TREATMENT. Vibriosis in pen-reared salmon is best controlled by prevention, and vaccines for this purpose are commercially available. Vaccination is carried out on fish that have attained immunocompetent size (5 -10 g) and before they are introduced to netpens. The vaccines are conveniently administered by immersion methods, and if applied properly, they afford excellent protection (Evelyn 1984; 1988b). Failures of protection in vaccinated fish, particularly with chinook, have occurred at a number of fish farms in B.C. The precise cause of these failures has not been determined, but the most likely explanation is that the fish may have been vaccinated before they were immunocompetent. Some farms have recently gone to revaccinating fish shortly after introduction to netpens, and intital results with revaccination in sea water have been promising. Revaccination should, however, be conducted with caution because the handling of fish shortly after their introduction to sea water may be very stressful. Revaccination by feeding, rather than by immersion, is being investigated because it would be a non-stressful means of administering the vaccine.

Antibiotics incorporated in feed are used to treat the disease once an outbreak has occurred. Presently, oxytetracycline at 100 mg drug/kg fish/day for 10 days is the most commonly used treatment for vibriosis in salmon. This treatment is usually efficacious if the disease is recognized early and fish are still actively feeding.

Furunculosis

Furunculosis is a septicemic disease caused by the Gram-negative bacterium *Aeromonas salmonicida* and excellent reviews on the disease and the causative agent are available (McCarthy and Roberts 1980; Paterson 1982; Austin and Austin 1987; Hastings 1988). The disease has long been recognized as a serious problem in freshwater-reared salmonids. With the increase in netpen farming, it has also become a serious disease of salmonids reared in sea water, particularly in Atlantic salmon. Like BKD, infected fish can carry the infection into sea water, and horizontal transmission can occur in netpens (Smith et al. 1982). The disease is favored by high or rising temperature, but it has occurred in netpens in the Pacific Northwest at temperatures as low as 6 °C. *Aeromonas salmonicida* has been reported from a large number of fishes, including marine species, and it is generally held that infected fish are the chief reservoirs of infection. This view is supported by the fact that fish may be cultured without the disease if water supplies to the culture facility are free of fishes that carry the pathogen. Notwithstanding this, survival of the pathogen outside of the host in fresh water, freshwater sediments, and sea water can be quite extensive (Rose et al. 1989, 1990). This property of the bacterium probably explains why horizontal infections in fresh and sea water occur, even when the sites involved are widely separated. The overwhelming body of evidence indicates that the bacterium is not transmitted from parent to progeny via the eggs (vertically transmitted).

Three subspecies of the bacterium are currently recognized (Popoff 1984), all of them having been reported as causing infections in salmonids. In pen-reared salmon in the Pacific Northwest, however, only one subspecies has thus far been encountered as a problem: *A. salmonicida* subsp. *salmonicida*. This subspecies produces a diffusing brown pigment when cultured on Tryptic Soy Agar, a property that helps with the diagnosis of furunculosis.

CLINICAL SIGNS AND GROSS PATHOLOGY. Fish with furunculosis may exhibit a wide spectrum of clinical and gross pathological changes, largely depending on whether the infection occurs in acute or chronic form. Early in more acute forms of the disease, fish may exhibit anorexia, darkening of the skin, and lethargy. Later, fish exhibit hemorrhages and reddening of the skin and fins. In more chronic cases, fish exhibit "furuncles" (i.e, vesicles containing serosanguinous fluid that underlie the skin) or large, bloody ulcers.

Internally, fish exhibit diffuse reddening, hemorrhages in the visceral organs, and enlargement of the spleen (Fig. 8, pg. 25).

MICROSCOPY. The most striking histological change in furunculosis is the lack of an inflammatory response to the infection. Colonies of the causative bacterium, associated with focal necrosis, are readily detected in the spleen, liver, kidney interstitium, and gills (Fig. 2).

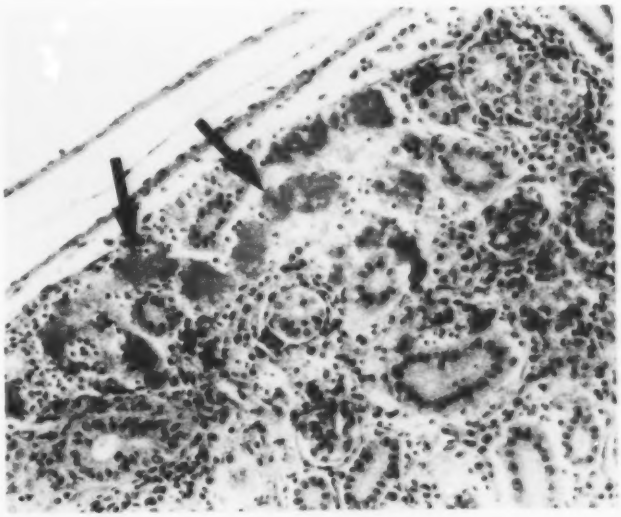


Figure 2. Aeromonas salmonicida *colonies (arrows) in the kidney interstitium of a coho salmon with furunculosis. H & E.*

DIAGNOSIS Diagnosis of furunculosis is achieved by isolation of *A. salmonicida* from the kidney or muscle lesions of fish with pathological changes characteristic of the disease. In addition, the presence of large, focal colonies of short bacilli in tissue sections can be used as a strong presumptive diagnosis.

The subspecies of *A. salmonicida* causing problems in pen-reared salmon in the Pacific Northwest grows readily on Tryptic Soy Agar, producing visible growth within 2-3 days at room temperature along with a characteristic brown pigment that diffuses into the growth medium (Fig. 9, pg. 26). The identity of the isolate can be confirmed serologically or phenotypically. Serological identification can be achieved using a latex bead agglutination kit (Microtek R & D, Ltd, Victoria, B.C.). The standard slide agglutination

technique cannot be used because the bacterium autoagglutinates (i.e., is self agglutinating in the absence of *A. salmonicida* antiserum). Phenotypic features diagnositic of the bacterium are as follows: it is a short (approximately 1 X 2 µm), Gram-negative, non-motile rod; ferments glucose with the production of acid and gas; is oxidase positive; and does not grow at 37 °C. Diagnosis of furunculosis can be expedited by using infected kidney tissues directly in the latex bead agglutination test. This technique can be used to by-pass the need for culturing the bacterium but it will only work with heavily infected tissue. In practice, however, diagnosis of furunculosis is usually done by isolation of the bacterium because one normally has to await the results of antibiotic sensitivity testing (which requires culturing the bacterium) before decisions on the antibiotic of choice for treating the infection can be made.

CONTROL AND TREATMENT. Furunculosis is a difficult disease to control. Infections may be chronic or subclinical, freshwater fish may carry the infection into sea water, and the disease is transmitted in sea water. A degree of protection against the disease can be achieved using vaccination. At this time, immersion-administered furunculosis vaccines are commerically available. Unfortunately, although these vaccines provide reasonable protection, the general view is that much more work is required before a truly satisfactory product is available (Hastings 1988).

Despite vaccination, farmers often have to resort to chemotherapy to control furunculosis. However, the pathogen often becomes resistant to antibiotics routinely used for treating the disease (Romet-30, oxytetracycline, and oxolinic acid) (Hasting and McKay 1987; Inglis et al. 1991), and outbreaks often recur shortly after treatment has terminated. In addition, mixed infections can occur, in which some fish are infected with antibiotic sensitive strains of *A. salmonicida,* while others from the same population are infected with resistant bacteria. Therefore, it is important to culture several fish in an affected population when determining the antibiotic of choice for treating a particular outbreak.

Probably the best way to control furunculosis is to avoid using smolts with a history of the infection, and to place these smolts on farms where they represent the only year class present. Unfortunately, these practices are often not followed in the Pacific Northwest because of the very high demand for Atlantic salmon smolts and because of the cost involved in operating separate sites for each year class.

Smolts free of the pathogen can be produced by using surface disinfected eggs and by raising the fry in water from a source that does not contain wild or cultured fishes. (Note: the pathogen is not transmitted within salmonid eggs; surface disinfection of the eggs is used only to avoid the possibility that the eggs may be surface-contaminated with the pathogen). As an additional precaution, apparently healthy smolts may be tested for absence of the bacterium prior to seawater introduction with a stress test (Bullock and Stuckey 1975b; McCarthy and Roberts 1980). The test facilitates detection of the pathogen in healthy "carrier" fish and involves injecting the fish with an immunosuppressive compound (e.g., prednisolone acetate) and holding the fish for 3 wk at 18 °C before attempting to isolate the bacterium.

Myxobacteriosis

Cytophaga and *Flexibacter* spp. are important bacterial pathogens of cultured fishes and usually cause external lesions in freshwater and marine species (Anderson and Conroy 1969; Pacha and Ordal 1970). *Flexibacter columnaris* and *C. psychrophila* are well recognized pathogens of fishes reared in fresh water (Pacha and Ordal 1970; Sniesko and Bullock 1976). In marine aquaculture, infections by *Flexibacter maritimus* have been observed in Japanese flounder and in sea breams (family Sparidae) in Japan (Baxa et al. 1986, 1987; Hikida et al. 1979; Masumura and Wakabayashi 1977; Wakabayashi et al. 1984, 1986) and Europe (Bernardet et al. 1990).

Proper taxonomic identifications have often not been conducted on many *Cytophaga* and *Flexibacter* spp. that have been associated with diseases in marine fishes. These bacteria are usually referred to as 'myxobacteria' by fish health workers and aquaculturists, but this is technically incorrect because these bacteria belong to the order Cytophagales, not the order Myxobacteria. It would be, therefore, more appropriate to refer to these bacteria collectively as cytophaga-flexibacter-like bacteria or gliding bacteria. However, to remain consistent with the common terminology and to avoid confusion, we continue to refer to these bacteria as myxobacteria in this text. Myxobacteria have been associated with skin lesions in seawater-reared salmonids for many years (Rucker et al. 1963; Borg 1960; Anderson and Conroy 1969; Wood 1974; Sawyer 1976). These bacteria have at times been identified as *Sporocytophaga* sp. However, the presence of microcysts (an

important diagnostic feature of this genus) has not been clearly demonstrated in these isolates.

Two types of myxobacterial infections have been associated with high mortality in pen-reared Atlantic salmon in the Pacific Northwest; one type causes large skin ulcers, and the other causes lesions primarily in the mouth. Myxobacteria infections are also seen in pen-reared Pacific salmon, but are not usually associated with severe epizootics. In Pacific salmon, myxobacteria are usually associated with frayed fins and erosion of the tail.

Myxobacterial skin lesions in Atlantic salmon

A *Cytophaga* sp. causes large skin lesions in Atlantic salmon smolts shortly after seawater entry (Kent et al. 1988a). Lesions and associated mortalities usually peak at about 1-3 wk after introduction to seawater, and based on our observations, the disease subsides after about 3-4 wk. There appears to be a seasonality to the disease, and fish introduced later in the spring and summer usually exhibit fewer skin lesions. Infections are restricted to the skin and underlying muscle. Fish with large lesions exhibit elevated plasma sodium levels (Kent et al. 1988a), which suggests that affected fish may ultimately die from an osmotic imbalance.

*Figure 3. Myxobacteria (*Cytophaga *sp.) in a wet mount from a skin lesion of an Atlantic salmon. (Courtesy of Dis. Aquat. Org.).*

CLINICAL SIGNS AND GROSS PATHOLOGY. Moribund fish exhibit large, white patches on the caudal peduncle and posterior region of the flanks when the dermis is intact (Fig. 9, pg. 27). Infected fish may also exhibit more severe lesions in which areas of the skin are completely destroyed and the underlying muscle is exposed (Fig. 9).

Figure 4. Myxobacteria (arrows) in the dermis and muscle of an Atlantic salmon with myxobacterial skin lesions. H & E. (Courtesy of Dis. Aquat. Org.).

MICROSCOPY. Wet mounts of the lesions reveal masses of filamentous bacteria (0.5-0.7 X 4-20 µm) (Fig. 3), which may exhibit gliding motility. Tissue sections of lesions where the skin is present reveals accumulations of filamentous bacteria on the surface of the skin, and throughout the epidermis and dermis. In more severely involved areas, large mats of filamentous bacteria replace the skin and bacteria invade deep into the underlying musculature (Fig. 4). Affected muscle is hemorrhagic and necrotic, but little inflammation is observed. The bacterium is observed only in the muscle and skin, and tissue sections of the gills and visceral organs have revealed no bacteria or significant pathological changes (Kent et al. 1988a).

DIAGNOSIS. Diagnosis of myxobacterial infections can usually be accomplished by observing large numbers of filamentous bacteria in wet mount preparations. Further identification of bacteria can be accomplished by culture on either Cytophaga Medium (see Appendix III) made with 50% sterile sea water or Marine Agar (Difco), and incubation at approximately 15 °C. Isolation of myxobacteria in pure culture may be difficult from skin lesions due to contamination with other faster growing bacteria (e.g., vibrios). However, the lesions usually contain very large numbers of the myxobacteria and serial dilutions of affected tissue in sterile 50% sea water will help to ensure the isolation of the bacteria in pure culture. The myxobacteria form small yellow-green, diffuse, rhizoid colonies on Cytophaga Medium (Fig. 9), and yellow-orange, smooth colonies with entire margins on Marine Agar (Fig. 9). More information on the characteristics of the myxobacteria isolated from Atlantic salmon skin lesions is found in Kent et al. (1988a). Reichenbach (1989) describes the characteristics of *Cytophaga* and *Flexibacter* spp. in general.

TREATMENT AND CONTROL. External treatments with antibiotics are often used to control myxobacterial infections in fresh water, but such treatments are not usually practical in netpens. These bacterial infections are often initiated in the skin where there are abrasions. Physical trauma during transport of smolts may allow the bacteria to establish an infection. Observations from fish farmers indicate that improved transport techniques and careful handling of fish greatly reduces the incidence of the disease.

Myxobacterial Stomatitis in Atlantic Salmon

Infections of the mouth and snout by myxobacteria have been observed in Atlantic salmon smolts during their first summer in sea water. The condition has occurred at many netpen sites and is often associated with high mortalities (Hicks 1990; Frelier et al. 1991). The infection appears to begin around the teeth. It has been suggested that the infection is initiated in periodontal tissue abraded by feeding on spiny crustaceans such as crab larvae and *Caprella* amphipods. Based on culture characteristics, the myxobacterium from mouth lesions appears to be a different organism than the myxobacterium causing skin lesions. A similar myxobacterial stomatitis has been observed in wild Atlantic cod in the North Sea (Hilger et al. 1991).

CLINICAL SIGNS AND GROSS PATHOLOGY. Infected fish are lethargic, emaciated, and anorexic, and some affected fish may exhibit flashing or head shaking. Early in the infection, examination of the mouth reveals focal, yellow bacterial mats around the palate and teeth, including the vomer (Fig. 9, pg. 27). The lesions may be single, but the opposing surface is often affected (Frelier et al. 1991). As the disease progresses, affected fish show multiple ulcers in the mouth with large bacterial mats overlying the lesions. The lesions may extend to the branchial arches and proximal esophagus, and the lower and upper jaw may be completely eroded in very severe cases. Severely affected fish do not feed.

MICROSCOPY. Wet mounts of the lesions reveal numerous filamentous myxobacteria. Histological examination of affected tissue reveals masses of the causative bacterium associated with focal ulcers, necrosis of the underlying bone, and a mixed inflammatory infiltrate (Frelier et al. 1991).

DIAGNOSIS. The disease can usually be diagnosed by observation of the distinctive yellow patches in the mouth of the affected fish. Positive diagnosis is achieved by observing filamentous bacteria in the mouth lesions.

CONTROL AND TREATMENT. There are unconfirmed reports that the disease can be controlled by feeding oxytetracycline. However, the treatment would have to be initiated before the fish cease active feeding.

Salmonid Rickettsial Septicemia

Diseases in salmonids due to obligate intracellular parasites, such as the rickettsias and chlamydias, are rare (see Cvitanich et al. 1991). A rickettsia has been shown to be the cause of severe septicemia in pen-reared salmon in Chile (Branson and Nieto Diaz-Munoz 1991; Cvitanich et al. 1991; Garcés et al. 1991). The name proposed by Cvitanich et al. (1991) for the disease in Chile is salmonid rickettsial septicemia, and Fryer et al. (1992) named the causative organism *Piscirickettsia salmonis*. In B.C., an essentially identical disease has been observed (see below). Because the causative organisms involved in Chile and B.C. are indistinguishable from each other morphologically, serologically, and culturally, and because the disease conditions in both locations are essentially identical, we have decided to adopt the names proposed for the Chilean disease and causative organism in B.C.

In B.C., the disease has been observed in pink salmon reared in seawater tanks at the Pacific Biological Station in 1970 and 1978, where it was associated with mortality. Subsequently, the disease was observed in pen-reared chinook (1983, 1984) and coho (1984) at this facility. In the chinook and coho, the disease was always incidental to some other more serious problem. As a result, salmonid rickettsial septicemia was regarded only with academic interest. In 1991, however, the disease was detected in chinook on three farms, and in Atlantic salmon at one of these farms. In the latter, the disease caused low level losses (Brocklebank et al. 1992).

Information to date on the epizootiology of the disease suggests that it is acquired in sea water from a marine source, that it is not vertically transmitted because it has never been observed in the freshwater rearing phase, and that it is transmitted directly from fish to fish (Cvitanich et al. 1991; T.P.T. Evelyn unpubl. data).

CLINICAL AND GROSS PATHOLOGY. Clinical and gross pathological changes associated with *P. salmonis* infections have been outlined by Cvitanich et al. (1991), Branson and Nieto Diaz-Munoz (1991) and Brocklebank et al. (1992). Affected fish are lethargic, anorexic, exhibit pallor of the gills due to anemia, are dark in color, and may swim near the surface. Multiple, small white spots and petechiae occur in the skin. Hallmark internal lesions are found in the liver. The livers of many affected fish exhibit large, whitish or yellow, multifocal, coalescing, pyogranulomatous nodules. These lesions often rupture, resulting in shallow crater-like cavities in the liver. Internal examination also reveals ascites, and an enlarged spleen and a gray, enlarged kidney. The spleen is extremely enlarged in infected pink salmon. Pallor (suggesting anemia) and petechiae are observed in the visceral organs and muscle, and a whitish pseudomembrane may cover the heart.

MICROSCOPY. Giemsa-stained imprints of infected tissues, especially the kidney and liver, reveal pairs or aggregates of basophilic coccoid organisms, about 0.5 - 1.5 µm in diameter. The organism is Gram-negative and is frequently found in the cytoplasm of macrophages (Fig. 5).

Cvitanich et al. (1991) and Branson and Nieto Diaz-Munoz (1991) described the histopathological changes in infected coho from Chile, and these changes are essentially the same as those seen in salmon from British Columbia (Brocklebank et al. 1992). The disease causes prominent tissue damage, and the liver, kidney, and spleen are the most severely affected organs. The liver exhibits large foci of necrosis and inflammation, and vasculitis and thrombosis. High magnification reveals aggregates of the organism in the cytoplasm of degenerated hepatocytes and macrophages, including melano-macrophages. Infected macrophages are usually hypertrophied and filled with cellular debris. The kidney interstitium exhibits generalized necrosis and prominent infiltration of macrophages. Vasculitis and thrombi are often found in the kidney (Fig. 5D). As with the liver, high magnification reveals the rickettsia in macrophages. These macrophages occur in large accumulations in the thrombi, or more diffusely throughout the kidney interstitium. The spleen exhibits similar histological changes as those seen in the kidney. In tissue sections stained with hematoxylin and eosin, the organism appears as basophilic or amphophilic spheres. Special stains showed that the organism is Gram, acid-fast, and PAS-negative, and they stain blue with Macchiavello's and toluidine blue stains (Brocklebank et al. 1992). We have found that the methylene blue stain is the best stain for showing the organism in tissue sections.

DIAGNOSIS. Presumptive diagnosis is achieved by observing the distinctive crater-like lesions and nodules in the liver. Reasonably definitive diagnoses can be achieved by observing the organism within phagocytic cells in Giemsa, Gram or methylene blue-stained imprints of the liver or kidney, or in macrophages in tissue sections along with the distinctive histological changes described above. Acridine orange-stained tissue smears are

also useful for demonstrating the organism (Lannan and Fryer 1991). Confirmatory diagnosis is based on isolation of the organism in cell culture using CHSE-214 cells (Fryer et al. 1990; Cvitanich et al. 1991), or by an indirect fluorescent antibody test (Lannan et al. 1991).

CONTROL AND TREATMENT. The organism is sensitive to various antibiotics in vitro, (Fryer et al. 1990; Cvitanich et al 1991), and oral treatment with oxytetracycline may have some benefit for controlling the disease. Because the disease is horizontally transmitted in sea water (Cvitanich et al. 1991), maintaining separate year classes at each site may reduce the prevalence of the infection by preventing transmission of the agent between year classes.

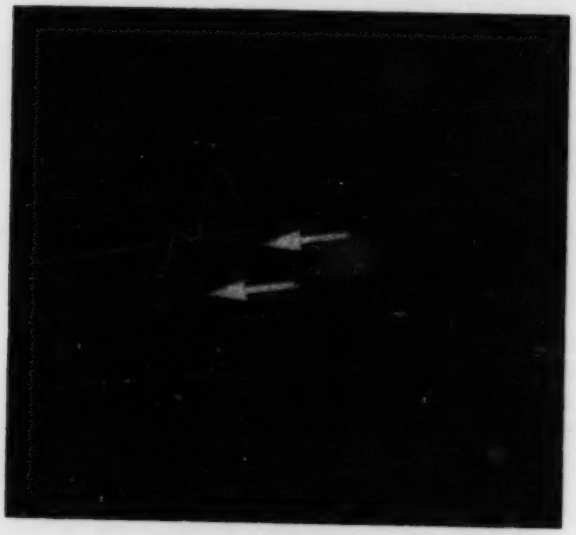


Figure 5A

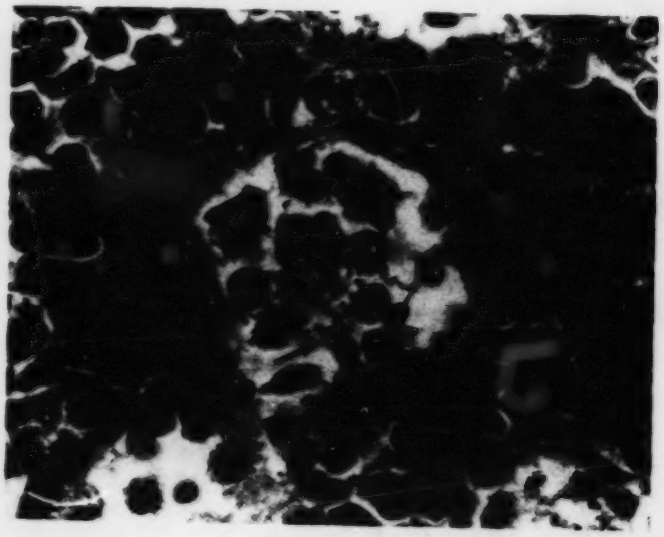


Figure 5B

Figure 5C

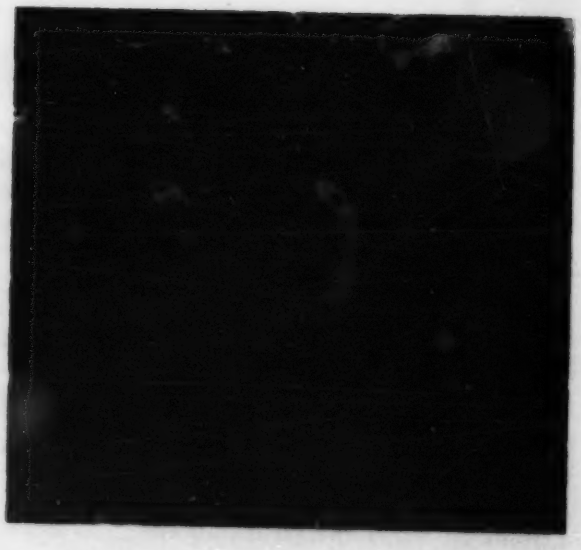


Figure 5D

Figure 5. Chinook with salmonid rickettsial septicemia. A. Gram Stain of kidney. Arrows = richettsia. B. Renal blood vessel with hypertrophied marcrophages with numerous rickettsia (arrows) in the cytoplasm. H & E. C. Focal necrosis (N) in the liver H & E. D. Thrombus (T) comprised primarily of infected macrophages in a renal blood vessel H & E.

VIRAL DISEASES

By G. S. Traxler and M. L. Kent

Several viruses are important pathogens of salmonid fishes, particularly during the early development of the fish in fresh water (Wolf 1988). Viral diseases of fishes have historically been of great concern to fish health managers because they can cause high mortality and because there are no vaccines or drugs available for their control. In addition, the mere presence of certain viruses in a population may cause economic hardship due to restrictions on transfer or sale of these fish.

Although viruses are serious pathogens of salmonids reared in fresh water, there have not been any losses attributable to known viruses among commercial netpen farmed salmon in the Pacific Northwest. Notwithstanding this, at least three viral diseases are potential threats for pen-reared salmon: infectious hematopoietic necrosis, infectious pancreatic necrosis, and viral erythrocytic necrosis. Plasmacytoid leukemia (PL), also known as marine anemia, of chinook may be the only viral disease that has caused significant losses in netpens in the Pacific Northwest. This disease may be caused by an oncogenic retrovirus (Kent et al. 1991a; Eaton et al. 1991). However, the etiology of PL has not yet been firmly established and this disease is, therefore, dealt with under the Idiopathic Diseases (see page 59).

Infectious Hematopoietic Necrosis (IHN)

Infectious hematopoietic necrosis virus is enzootic in fresh water along the Pacific coast of North America, and the virus has been spread to Asia and Europe. Originally the virus was associated with losses among chinook, sockeye, kokanee, rainbow trout, and steelhead trout populations. The host range of the virus has, however, since been expanded because natural infections have been reported among brown trout, and Atlantic, chum, pink, amago, and masou salmon (Wolf 1988.). Experimentally, brook trout and cutthroat trout have been shown to be susceptible to IHN virus infection. Recently, there have been several isolations of the virus from adult coho, a species previously considered to be refractory to IHN. However, the isolations from coho were not associated with losses (LaPatra et al. 1989). Initially, IHN was considered to be a significant disease only in very young fish. However, certain chronic affects, such as scoliosis have been attributed to the virus (Amend et al. 1969).

The virus is readily transmitted by waterborne exposure of susceptible fish. In addition, there is some evidence indicating that vertical transmission via the reproductive products occurs (Mulcahy and Pascho 1985), but it is generally believed to be an infrequent event (LaPatra et al. 1990).

Outbreaks of IHN have not been observed in netpens. However, experimental seawater transmission to sockeye and Atlantic salmon post-smolts has been demonstrated by both waterborne exposure of the virus and by cohabitation with IHN-infected fish (Traxler et al. 1991b). The marked susceptibility of post-smolt Atlantic salmon in this experiment indicates that IHN is a potential problem for pen-reared Atlantic salmon.

CLINICAL SIGNS AND GROSS PATHOLOGY. Swimming behaviour can vary from lethargy to rapid erratic flashing motions. Externally, affected fish are usually dark and exhibit hemorrhages at the base of the fins. The gills and liver may be pale, indicating anemia, and affected fish show ascites, and petechial and ecchymotic hemorrhages throughout the viscera (Fig. 9 pg. 27). The digestive tract may be filled with a yellowish mucus-like fluid.

MICROSCOPY. The hematopoietic tissues of the kidney and the spleen are the most severely affected (Yasutake 1970). In the early stages of infection these tissues exhibit focal necrosis, which progresses to severe, diffuse necrosis and degeneration of these tissues (Fig. 6). The gills, pancreas, liver, and submucosa of the alimentary tract may also exhibit diffuse necrosis.

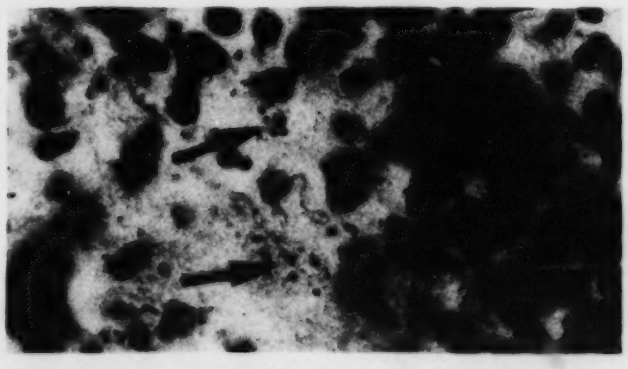


Figure 6. Tissue section of the kidney of a sockeye salmon with IHN. Note severe necrosis in the hematopoietic cells (arrows) in the kidney interstitium. H & E.

DIAGNOSIS. Presumptive diagnosis of IHN can be achieved by observing severe, diffuse necrosis in the hematopoietic tissue, which can be detected in histological sections or tissue imprints (Yasutake 1978). At present, immunodiagnostic or molecular biology tests (e.g., DNA probes) for fish viruses are not commercially available, and detection of the IHN virus infection is dependent upon growing the virus in tissue culture, where cell damage or cytopathic effects (CPE) can be observed in the cultured cells. Diagnosis of the disease is usually based upon isolation of the virus in tissue culture, accompanied with the observation of necrosis in the target tissues in the affected fish. The most commonly used cell lines for IHN virus isolation are EPC (epithelioma papillosum cyprini), CHSE-214 (chinook salmon embryo), and FHM (fathead minnow) with an incubation temperature of 15 °C. Confirmatory diagnosis of IHN virus is usually accomplished by neutralization of the isolate with specific antisera, or by a fluorescent antibody assay (Winton 1991).

The gross pathological changes in Atlantic and sockeye salmon experimentally infected with IHN virus in sea water (Fig. 9) were indistinguishable from those caused by Gram-negative bacterial septicemias (Traxler et al. 1991b). Therefore, diagnosticians should consider conducting virus assays on fish with generalized internal hemorrhaging that are not consistently infected with known bacterial pathogens.

CONTROL. Infectious hematopoietic necrosis is controlled by avoiding the infection. In freshwater hatcheries, this is usually accomplished using eggs from IHN virus-free females, disinfecting eggs with iodophores, and rearing eggs and fry in IHN virus-free water (Winton 1991). Rearing Atlantic salmon and chinook in netpens in freshwater lakes before seawater entry is becoming a popular practice in the Pacific Northwest. The virus is prevalent in sockeye salmon in some of these lakes, which could be a potential source of transmission to pen-reared fish. Therefore, until the risk of transmission of IHN virus is assessed in this situation, we suggest that these lakes be avoided or that fish reared in such pens be tested for the presence of the virus before they are transferred to sea water. In addition, only smolts free of the virus should be transported to netpen sites because the disease can be transmitted in sea water.

Infectious Pancreatic Necrosis (IPN)

The virus (several serotypes) that causes infectious pancreatic necrosis in cultured salmonids belongs to the family Birnaviradae. Viruses in the Birnaviradae have a widespread geographic and host range with isolations reported from numerous species of freshwater and marine fishes, and from many species of marine invertebrates (McAllister 1983). However, IPN-like isolates from non-salmonids are usually non-pathogenic for salmonids. The classification of this group of viruses is complex and their organization is dependent upon the methods used for comparison (Caswell-Reno et al. 1989).

The most susceptible species of fish appear to be rainbow trout, brook trout, brown trout, and Atlantic salmon. Younger fish usually suffer acute infections resulting in losses often exceeding 90%. Fish over 6 months old are usually more resistant to IPN infections. Survivors of IPN outbreaks may become carriers, shedding virus throughout their life and acting as a source of re-infection. The infection is prevalent in pen-reared Atlantic salmon in Norway in which it has recently been associated with severe losses (T. Poppe, National Veterinary Institute, Oslo, Norway, pers. comm.). Vertical transmission of IPN virus has been well documented (Wolf et al. 1963; Bullock et al. 1976; Fijan and Giogetti 1978), and has occurred in groups of eggs that had been treated with iodophores.

In 1989 a birnavirus was isolated from a group of Atlantic salmon post-smolts from a seawater netpen site in British Columbia. Tests conducted to identify the isolate concluded that the virus was most similar to, but not identical with, a strain of IPN virus isolated from brook trout at Jasper, B.C. (Kieser et al. 1989). This isolate, referred to at the time as IPN virus, proved to be non-pathogenic to fry of chinook, coho, and Atlantic salmon, and rainbow trout (Traxler and Evelyn 1991). In view of the failure of the isolate to cause IPN in the tested salmon, the virus would more appropriately have been identified as a birnavirus than IPN virus.

CLINICAL SIGNS AND GROSS PATHOLOGY. The initial sign of IPN is often a sudden increase in mortality. Moribund fish may be unable to swim against the current, are usually dark in color, and show abdominal distension and exophthalmus. Internal organs are often pale, and the digestive tract is often devoid of food and filled with a whitish mucus due to sloughing of the intestinal epithelium (Wolf 1988).

MICROSCOPY. As the name implies, IPN virus causes necrosis of the exocrine pancreas. Intracytoplasmic inclusions have been reported (Yasutake 1970). Lesions have also been reported in the hematopoietic and excretory tissues of the kidney, the mucosal cells of the digestive tract, and the liver parenchymal cells (Wolf 1988).

DIAGNOSIS. Virus isolation accompanied with the described pathological changes in the target tissues are the basis for diagnosis of IPN. The cell lines of choice are RTG-2 and CHSE-214 and they should be incubated at a temperature of 15 °C. The use of two or more dilutions of the inoculum are recommended in order to quantify the virus present. This approach allows the diagnostician to determine whether carrier or lethal levels of the virus are present. It also helps to reduce toxicity of the tissue extracts for the cell cultures and helps to avoid inhibition of IPN virus due to high concentrations of fish tissue homogenates (Dixon 1987). Positive identification of the virus is accomplished by serological methods such as serum neutralization or fluorescent antibody techniques.

CONTROL. Because IPN virus can be vertically transmitted and infected fish can excrete virus for the rest of their life, the only effective control method is avoidance. The use of IPN virus-free broodstock, rearing progeny in virus-free water, and restricting the movement of fish can reduce the spread of IPN virus.

Viral Erythrocytic Necrosis (VEN)

Viral erythrocytic necrosis (VEN) has been reported in various species of marine and anadromous fishes throughout the world (Appy et al. 1976; Walker and Sherburne 1977). Along the Pacific coast of North America, natural occurrences of VEN have been documented in chum, pink, coho and chinook salmon, steelhead trout, and Pacific herring (Bell and Traxler 1985; Meyers et al. 1986; Rohovec and Amandi 1981).

The viruses responsible for VEN have been tentatively placed in the family Iridoviradae. Virions observed from fish are icosahedral with a diameter ranging from 140-350 nm. Most of the virions found in Pacific herring and salmon fall within the 140-210 nm size range, whereas those reported from Atlantic cod ranged from 310-360 nm in diameter (Appy et al. 1976).

The widespread range of VEN viruses and susceptibility of salmonids to at least one of these viruses suggest that the virus poses a threat to netpen-cultured salmonids. Viral erythrocytic necrosis was frequently observed among pink and chum salmon farmed in netpens located at Departure Bay, B.C. but coho, chinook, and sockeye salmon raised at the same site did not appear to acquire the disease (Evelyn and Traxler 1978). However, a survey of anadromous salmonids in Oregon revealed that 25% of the populations tested were positive for VEN virus (Rohovec and Amandi 1981). Positive species were spawning adult coho, chinook, and chum salmon, and steelhead trout.

Natural occurrences of VEN in Pacific herring in Alaska resulting in high losses were reported by Meyers et al. (1986). The VEN virus from Pacific herring has been transmitted to chum, pink, and Atlantic salmon using intraperitoneal injection, and the virus in the salmon was associated with anemia and death (Evelyn and Traxler 1978; MacMillan and Mulchay 1979; Traxler et al. 1991a). Preliminary evidence from transmission studies conducted in our laboratory also indicate that VEN virus from Pacific herring is transmittable to Atlantic salmon by cohabitation with infected herring in sea water.

CLINICAL SIGNS AND GROSS PATHOLOGY. In heavily infected fish the most significant clinical sign is severe anemia, indicated by pallor of the gills and visceral organs. The virus infects erythrocytes, resulting in reduced hematocrit values (Evelyn and Traxler 1978). Some fish may exhibit unilateral or bilateral exophthalmus (MacMillan et al. 1989). Infection with VEN virus usually results in chronic, low level losses. A significant side-effect of VEN is reduced resistance to other pathogens and environmental stressors (Macmillan and Mulcahy 1979; Macmillan et al. 1980).

MICROSCOPY. Histological features of VEN are not pathognomonic. The kidney may show increase in hematopoiesis. The characteristic cytoplasmic inclusions in erythrocytes that accompany the infection are not frequently observed in tissue sections. Blood smears stained with Giemsa typically reveal eosinophilic or amphophilic intracytoplasmic inclusions (0.8 - 4.0 µm in diameter) in erythrocytes (Fig. 10, pg. 28). In chum salmon, erythroblasts are also infected as the disease progresses (MacMillan et al. 1989). Affected cells usually contain a single round or oval inclusion in the cytoplasm. Transmission electron microscopy of infected erythrocytes reveals large, spherical viroplasms and pentagonal or hexagonal virions (about 150 - 200

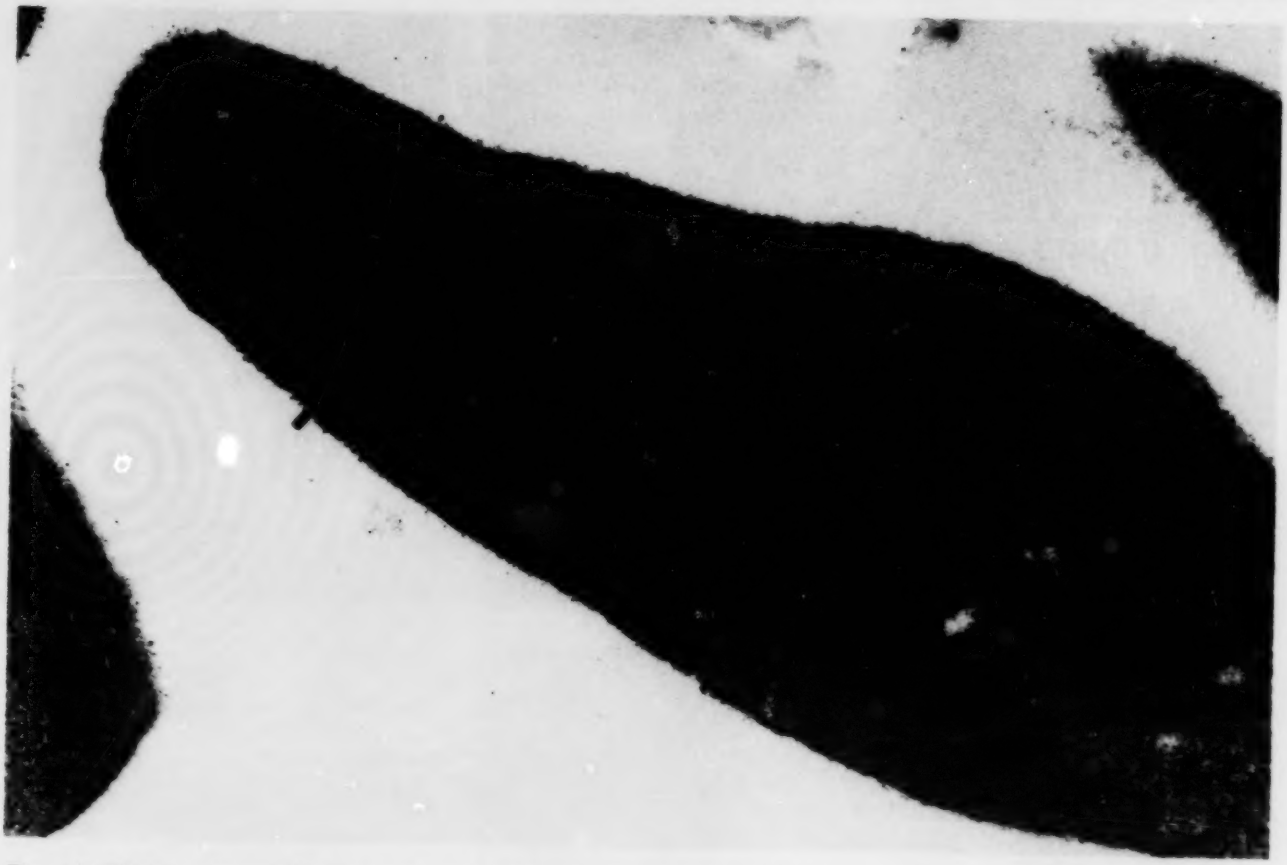


Figure 7. Electron micrograph of an erythrocyte of Atlantic salmon infected with VEN virus (arrows). V = viroplasm, N = nucleus.

nm) in the cytoplasm (Fig. 7). The viroplasm likely represent the inclusions observed in Giemsa-stained blood smears.

DIAGNOSIS. The viruses responsible for VEN have not been grown in cell culture. A presumptive diagnosis is made by finding the characteristic intracytoplasmic inclusions in erythrocytes. Confirmation is accomplished by observing the characteristic virions by electron microscopy.

CONTROL. There are no known control measures. Horizontal transmission has been well documented, making avoidance difficult given the widespread marine distribution of the virus. There is evidence that individual fish are able to recover from VEN. Maintaining affected stocks in conditions of low stress may reduce losses.

FUNGI

Two fungi, the rosette agent and *Ichthyophonus* (presumably *I. hoferi*) have been recognized as causes of disease in pen-reared salmon in the Pacific Northwest.

Rosette Agent

A severe infectious disease of chinook reared in netpens has occurred repeatedly at the U.S. National Marine Fisheries Service experimental station in Manchester, Washington, USA (Elston et al. 1986; Harrell et al. 1986). The disease is caused by an unclassified intracellular protistan parasite with affinities to colorless algae or fungi. The parasite primarily infects macrophages in the spleen and kidney, but it may occur in other organs in heavy infections. Mortality due to the disease is highest in the summer and fall, and losses of over 90% have occurred in some years. Epizootics caused by the pathogen have been only observed in 3-year-old chinook during their second summer in sea water (Harrell et al. 1986), but younger chinook can be experimentally infected (Elston et al. 1986). Elston et al. (1986) isolated the organism in culture and found that it grew only in the CHSE-214 cell line, which is derived from chinook. The authors concluded that the organism is an obligate intracellular parasite of chinook, but the source of infection was not determined. A similar, probably identical organism, has been reported in Atlantic salmon reared in fresh water in California (Hedrick et al. 1989) and in seawater-reared Atlantic salmon on the Atlantic coast of Canada (Cawthorn et al. 1991).

CLINICAL SIGNS AND GROSS PATHOLOGY. Infected fish are anemic, and exhibit an enlarged spleen and kidney.

MICROSCOPY. Histological examination of heavily infected spleens and kidneys reveals numerous eosinophilic spherical organisms in phagocytic cells associated with multifocal necrosis. The organism can readily be identified from heavily infected fish in Giemsa-stained imprints of the spleen and kidney. In imprints, the parasites appear as 3-7 µm spheres surrounded by a distinct clear halo (Fig. 10, pg. 28). They are often found in clusters or rosettes within macrophages, hence the name rosette agent.

DIAGNOSIS. The infection is diagnosed by detecting the parasite in either histological sections or Giemsa-stained imprints of the kidney or spleen.

CONTROL AND TREATMENT. There are no known treatments for rosette agent infections.

Figure 8A

Figure 8B

Figure 8. Bacterial diseases. A. Pale gills due to anemia in a coho salmon with bacterial kidney disease (BKD). B. Coho salmon with BKD. The kidney is enlarged and has focal, white, granulomatous lesions, and a pseudocapsule covers the enlarged spleen. C,D,E. Chinook salmon smolts with vibriosis. C. Hemorrhages in the skin on the head and operculum. D. Multifocal hemorrhages in liver. E. Large, coalescing hematomas (H) in the liver. F. Atlantic salmon with furunculosis. The visceral organs and peritoneal lining exhibits extensive hemorrhaging, and the lower intestine is enlarged. (Courtesy of R.A. Elston).

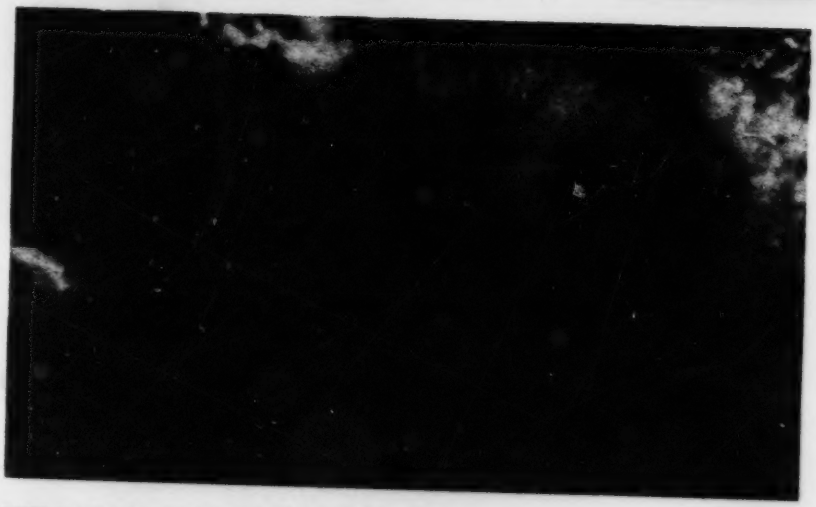


Figure 8C

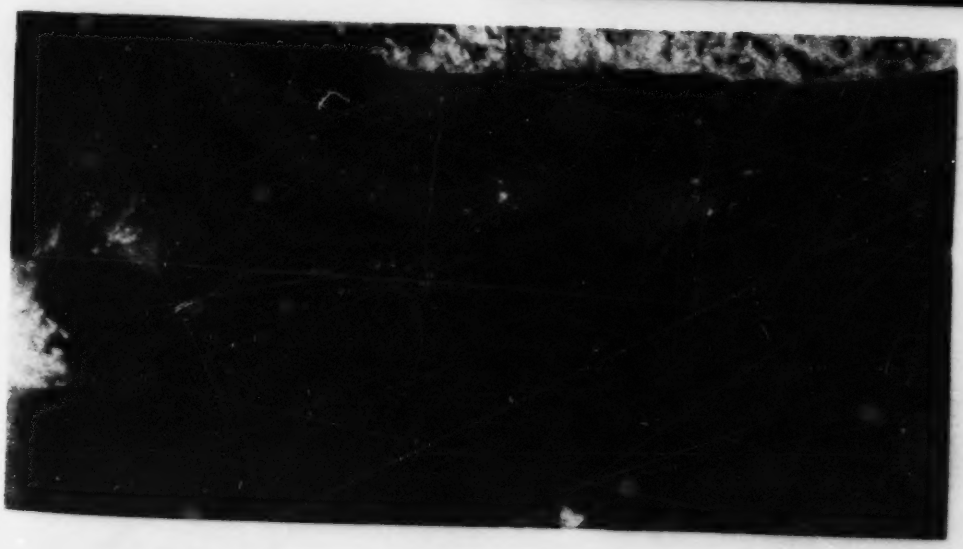
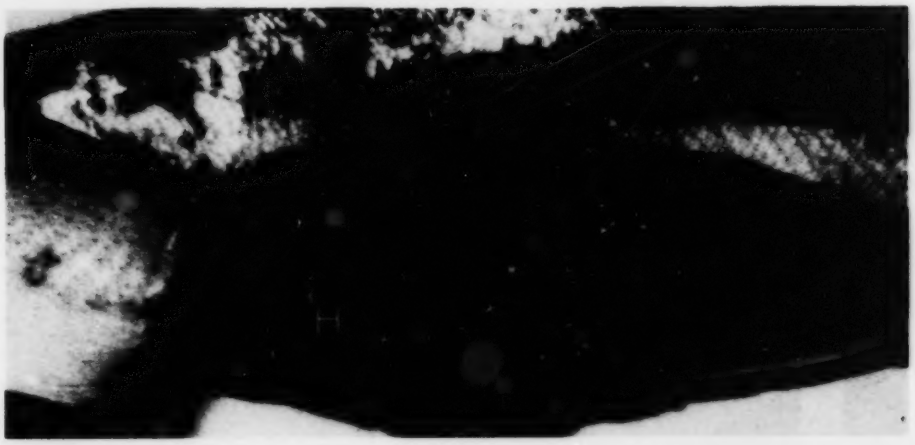


Figure 8D



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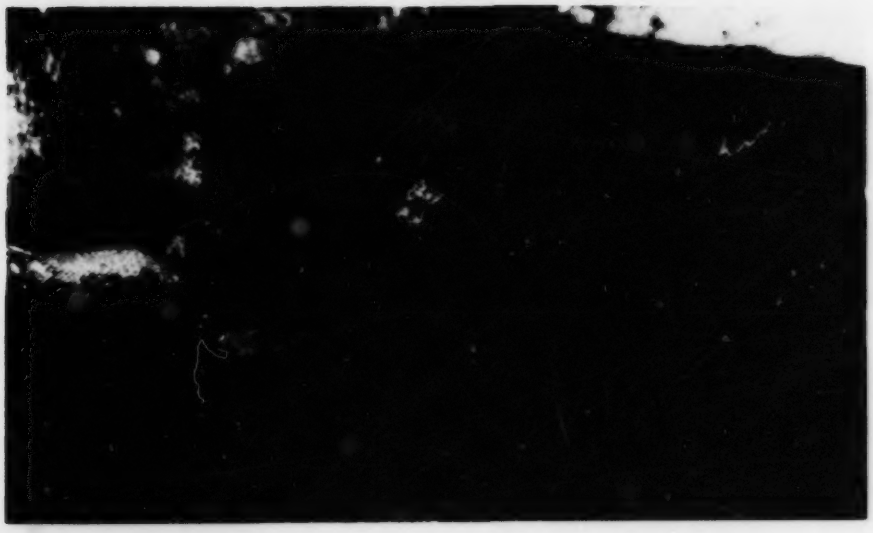


Figure 8F

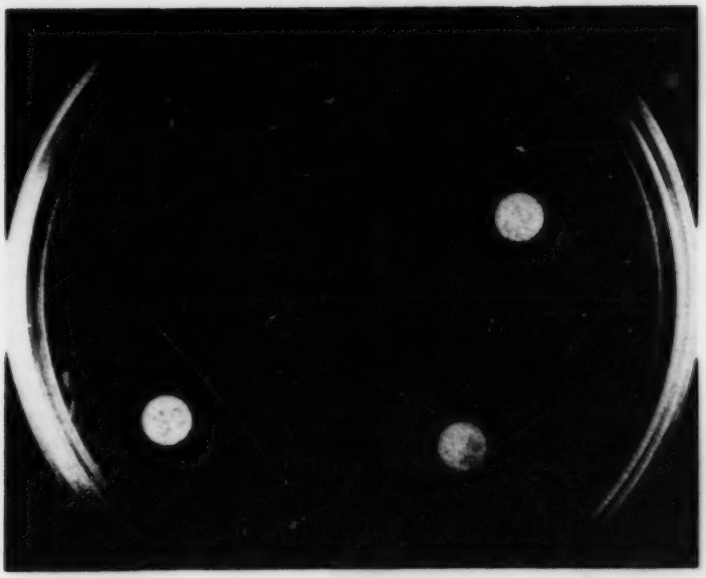


Figure 9A

Figure. 9. Bacterial and viral diseases.
A. Aeromonas salmonicida *grown on Tryptic Soy Agar. Note the diffusible, brown pigment.*
B, C. Myxobacteria (Cytophaga *sp.) from Atlantic salmon skin lesions. B. Bacteria grown on Seawater Cytophaga medium. C. Bacteria grown on Marine Agar (Difco). D, E. Atlantic salmon with myxobacterial infections. D. Skin infection; the dermis is exposed on the flanks and the underlying muscle is exposed (arrow) at the caudal peduncle. E. Mouth infection; note focal yellow nodules (arrows). F. Infectious hematopoietic necrosis (IHN) in an Atlantic salmon experimentally infected in sea water. Extensive hemorrhages occur in the viscera and muscle.*

Figure 9B

Figure 9C

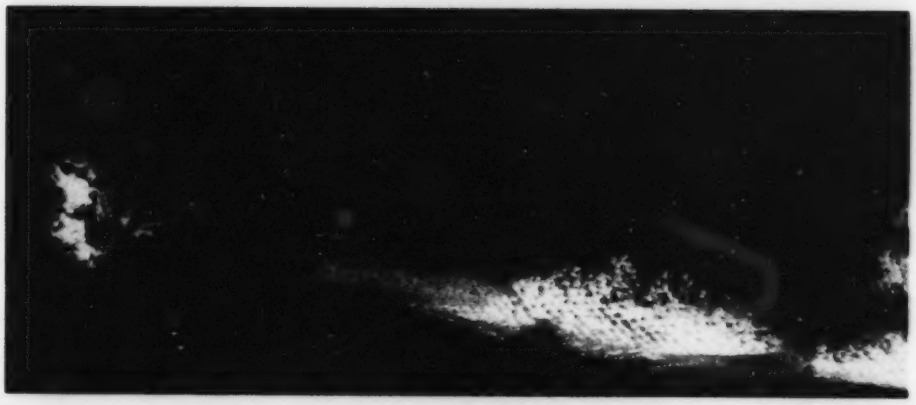


Figure 9D

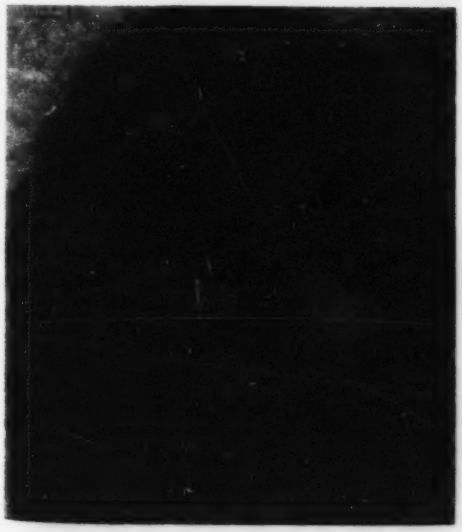


Figure 9E

Figure 9F

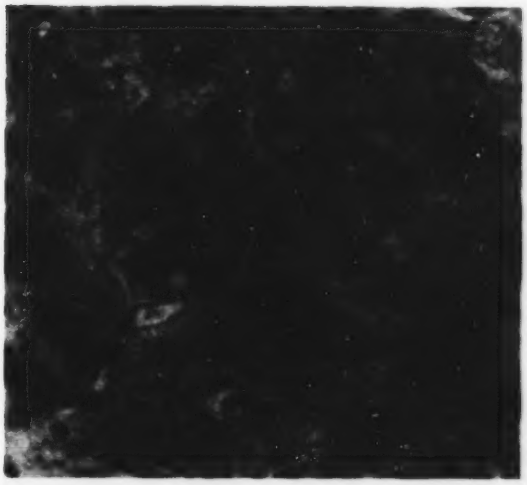


Figure 10A

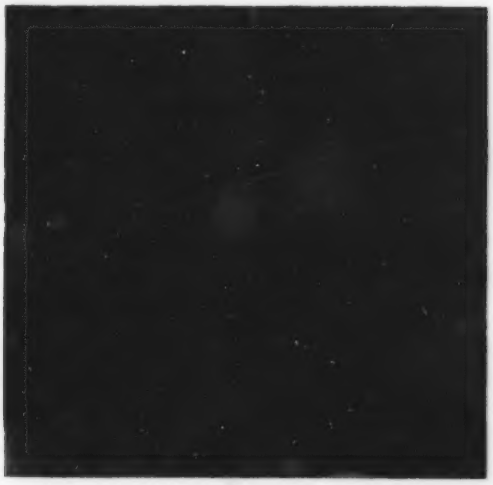


Figure 10B

Figure 10C

Figure 10D

Figure 10E

Figure 10F

Figure 10. A. Tissue section of chinook kidney with BKD stained with Periodic acid-Schiff (PAS). Note PAS-positive (red) bacteria in phagocytes in the kidney interstitium. B-D. Pathogenic bacteria in kidney imprints stained with Gram stain. B.* Renibacterium salmoninarum *from salmon with severe BKD. C.* Vibrio anguillarum *from chinook with* vibrosis. *Note curved rods (arrows). D.* Aeromonas salmonicida *(arrows) from Atlantic salmon with furunculosis. E. Blood smear of pink salmon with VEN. Arrow = cytoplasmic inclusions. Leishman's Giemsa. F. Rosette agent (arrows) in imprint from chinook kidney. Note clear halo around the spherical fungi (arrows). Leishman's Giemsa.

Ichthyophonus hoferi

Ichthyophonus hoferi is a common fungal pathogen of many species of wild marine fishes (McVicar 1982; Sinderman 1990). The infection is very prevalent in some species, and the fungus has caused severe disease and mortality in some fishes, such as Atlantic herring (Sinderman 1990) and plaice (McVicar 1982). The fungus also causes disease in freshwater species. Infections have been reported in freshwater-reared rainbow trout, apparently the result of feeding infected marine fish. Salmonids are very susceptible to the infection, and McVicar (1982) warned that salmon would be vulnerable to the infection when reared in netpens. The fungus is spread from fish to fish by ingestion of spores or infected fish.

We have observed heavy infections in Atlantic salmon smolts in their first summer in sea water. It is unclear how and when the smolts became infected. However, Atlantic salmon smolts often feed heavily on natural marine organisms, and the infected fish were feeding on calinoid copepods about a month before the infection was detected. Some have suggested that the fungus is spread in infected copepods, and it is possible that the smolts became infected by feeding on infected copepods or another marine organism.

CLINICAL SIGNS AND GROSS PATHOLOGY. Infected Atlantic salmon smolts from netpens in B.C. were lethargic and extremely emaciated–clinical signs that have also been reported in other fish infected with *I. hoferi* (McVicar 1982). Infected salmonids may also show curvature of the spine (McVicar 1982). Dissection of heavily infected fish reveals focal, white granulomatous lesions in the visceral organs, particularly the heart. Infections of the skeletal muscle may reduce flesh quality. Although poor flesh quality has not been associated with the infection in salmon, this condition has been observed in non-salmonid fishes (McVicar 1982).

MICROSCOPY. Squash preparations or histological sections of infected organs reveal the thick-walled resting spores (Fig. 11), which is the most commonly observed developmental stage in fish. The spores are variable in size and may reach 100-200 µm (McVicar 1982). The spores are Periodic acid-Schiff (PAS) positive and are multinucleated. After the host dies, the spores produce a germination tube and branched hyphae (Fig. 12). In plaice and haddock, the spores germinate 15-30 min after death at 20°C (McVicar 1982).

The tissue reaction to *Ichthyophonus* infections is characterized by granuloma formation around the resting spores (Fig. 11). Infections are often most severe in the heart, but essentially any visceral organ, the skeletal muscle, and the brain may be infected.

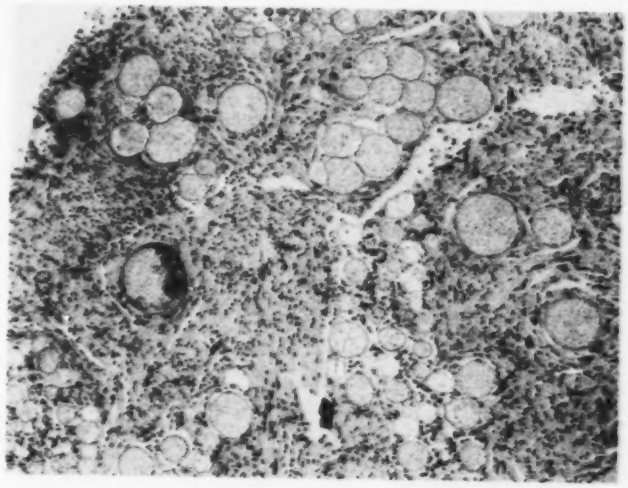


Figure 11. Multinucleate Ichthyophonus *spores in the heart of an Atlantic salmon. H & E.*

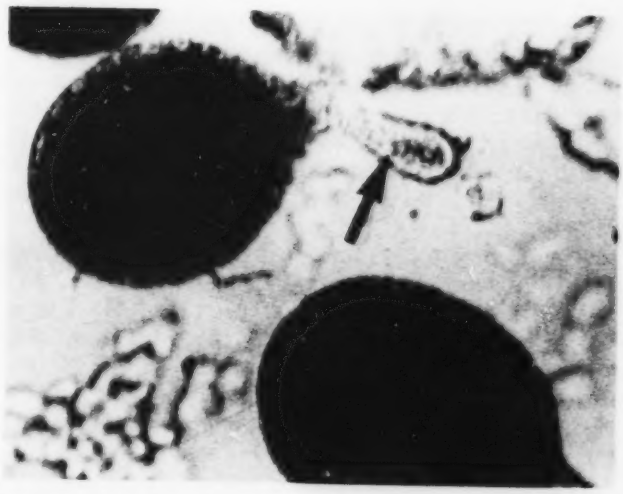


Figure 12. Wet mount preparation of Ichthyophonus *spore. Arrow = germination tube. (Courtesy of the Registry of Study Materials of the Charles Louis Davis, D.V.M. Foundation for the Advancement of Veterinary & Comparative Pathology).*

DIAGNOSIS. The infection can be presumptively diagnosed in squash preparations by finding the spherical bodies of varying size ranging up to 200 µm. Confirmatory diagnosis can be achieved by

observing post mortem germination of the resting spores or by observing the multinucleate resting spores, with a thick fibrous capsule, in histological sections. The fungus has been cultured in vitro, but this is not required for identification because the large resting spores are distinct from other fungi of fishes.

CONTROL AND TREATMENT. Van Duijin (1956) indicated that fungicidal drugs such as phenoxyethanol might be effective for treating early infections. However, there are no commercially available therapeutants for *Ichthyophonus* infections, and the disease is generally considered incurable. Salmon in netpens may become infected by feeding on natural marine biota (e.g., Pacific herring or copepods). If this is indeed the case, it would be very difficult to avoid the infection in the netpen environment.

PROTOZOAN PARASITES

Protozoans are important pathogens of cultured fishes, and several have caused significant diseases in pen-reared salmon in the Pacific Northwest (Fig. 13). An amoeba, *Paramoeba pemaquidensis*, and a flagellate, *Ichthyobodo* (= *Costia*) sp., infect gill surfaces of salmon. A systemic infection of a diplomonad flagellate, similar to *Hexamita salmonis*, has caused high mortality in chinook. Two myxosporeans, *Parvicapsula* sp. and *Kudoa thyrsites*, and two microsporidians, *Loma salmonae* and *Enterocytozoon salmonis*, infect internal organs. In addition, other species known to infect salmonids in freshwater hatcheries and natural waters may ultimately be found in pen-reared salmon.

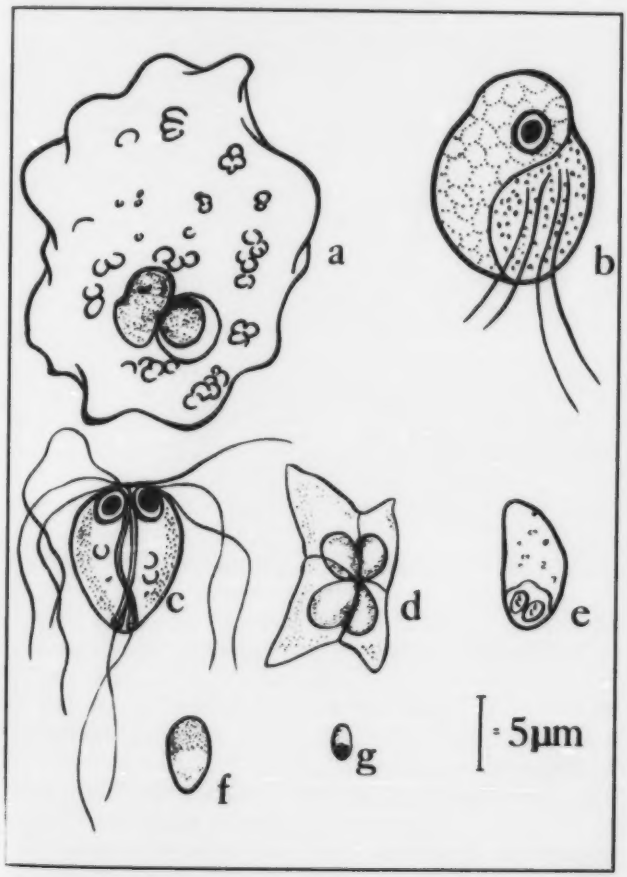


Figure 13. Protozoan parasites of seawater-reared salmon in the Pacific Northwest: a, Paramoeba pemaquidensis*; b,* Ichthyobodo necator *(=*Costia necatrix*); c,* Hexamita salmonis*; d,* Kudoa thyrsites*; e,* Parvicapsula *sp.; f,* Loma salmonae*; g,* Enterocytozoon salmonis*. Bar = 5 µm.*

Gill Amoeba *Paramoeba pemaquidensis*

Paramoeba pemaquidensis(Sarcomastigophora: Paramoebidae) has been associated with severe gill disease in coho reared in netpens in Washington State and land-based seawater tanks in California (Kent et al. 1988c). A similar, possibly identical, amoeba has caused devastating losses in pen-reared rainbow trout and Atlantic salmon in Tasmania (Munday et al. 1992; Roubal et al. 1989). *Paramoeba pemaquidensis* is an opportunistic pathogen that is normally free-living in sea water. Intensity and prevalence of the amoeba on fish gills has varied from year to year, with infections being most prevalent in the late summer and fall. At one farm in Washington, 25% mortality was attributed to the amoeba in 1985. The exact environmental conditions or health status of the fish that allow the organism to proliferate on fish gills are unknown. Presumably fish already compromised by other diseases are more susceptible to the infection, and at times heavy infections are observed on fish debilitated by preexisting disease or smoltification problems.

CLINICAL SIGNS AND GROSS PATHOLOGY. Consistent with respiratory problems of fish, heavily infected fish are lethargic, accumulate at the surface, and have flared opercula. Excessive mucus is often observed on heavily infected gills. Focal, whitish patches may be observed on gills of heavily infected fish (Fig. 20, pg. 38).

MICROSCOPY. Floating and transitional forms of the amoeba on the gills are 20-30 µm in diameter and have several digitiform pseudopodia (Fig. 14). Careful observation reveals movement in the amoebae. In wet mounts, amoebae attach to the slide after about an hour, resulting in a locomotive form measuring approximately 20 X 25 µm. *Paramoeba* spp. contain a unique structure, called a parasome or Nebenkörper, which is adjacent to the nucleus. The parasome can be observed in wet mounts of locomotive forms, and is readily visible with Feulgen DNA stains (Fig. 14).

Histological sections of affected gills reveal prominent epithelial hyperplasia and fusion of the distal portion of secondary lamellae, which often results in formation of large interlamellar vesicles (Fig. 14). The amoebae are usually confined to the gill surface and rarely penetrate the epithelium.

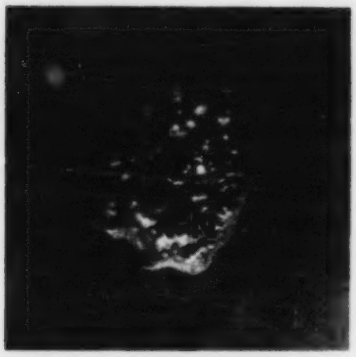


Figure 14A

Figure 14B

Figure 14C

Figure 14D

Figure 14. Paramoeba pemaquidensis *from coho salmon gills. Arrow = parasome, N = nucleus. A,B. Wet mounts of locomotive form (A) and transitional form (B). Nomarski phase contrast. C. Feulgen stain of amoeba. D. Gill with hyperplasia associated amoeba (arrows) infection. H & E. (Courtesy of Dis. Aquat. Org.).*

DIAGNOSIS. Paramoebiasis of salmon is diagnosed by the detection of large numbers of the amoebae on the gills. The organisms are best identified in fresh wet mount preparations of the gills. Amoebae can also be identified on gill surfaces in histological preparations, but many will fall off the gill surfaces during processing.

TREATMENT AND CONTROL. Munday et al. (1992) found that most compounds typically used as external treatments (e.g., formalin, chelated copper, diquat, malachite green and chloramine T) did not eradicate the disease, but they found that the amoeba is quickly eradicated from fish gills with freshwater bath treatments. The organism requires sea water for growth and survival, and grows poorly at salinities below 10 ppt (Kent et al. 1988c). Reducing the salinity has been effective for eradicating infections in fish held in land-based tanks, but this treatment is usually difficult to apply and impractical in netpens.

Systemic diplomonad infection in chinook

Severe systemic infections by a diplomonad flagellate (family Hexamidae) resembling *Hexamita salmonis* caused close to 50% mortality in chinook at one netpen site in the Sechelt area. The fish were introduced to sea water in the spring of 1990 and showed unusual mortality starting in September 1991. *Hexamita salmonis* is a common parasite of the intestinal tract of salmonids reared in fresh water. Most infections do not cause disease, but some reports have attributed anorexia, emaciation, poor growth and mortality in salmon fry to the infection (Davis 1961; Sano 1970; Becker 1977). Other diplomonad parasites infect strictly marine fish (Poynton and Morrison 1990), and Lom (1984) suggested that *H. salmonis* may persist in salmonids after they migrate to sea water. Systemic infections by diplomonad parasites in fish are rare and we are aware of only two other reports of such infections; Mo et al. (1990) described systemic hexamitosis in pen-reared Atlantic salmon from Norway, and Ferguson and Moccia (1977) reported a similar disease in Siamese fighting fish. Although the flagellate observed in the blood of chinook is morphologically indistinguishable from the relatively non-pathogenic *H. salmonis* that infects the intestinal tract of salmonids in fresh water, it may represent a new, highly invasive strain or species. In both fresh water and seawater aquaria, we could readily reproduce the systemic disease in chinook by water-borne exposure of the fish to infected blood and viscera, or by cohabitation with infected fish.

CLINICAL AND GROSS PATHOLOGY. Externally, infected fish appear normal except some fish exhibit a distended, swollen abdomen. The gills are pale due to anemia. The hallmark gross pathologic change of the disease is an extremely hypertrophied liver (Fig. 20, pg. 38). The liver may also be mottled, and have petechial hemorrhages and whitish, friable areas. Affected fish consistently exhibit serosanguinous ascites and blood clots in the visceral cavity (Fig. 20). The spleen and kidney are moderately enlarged, and petechiae occur throughout the skeletal muscle.

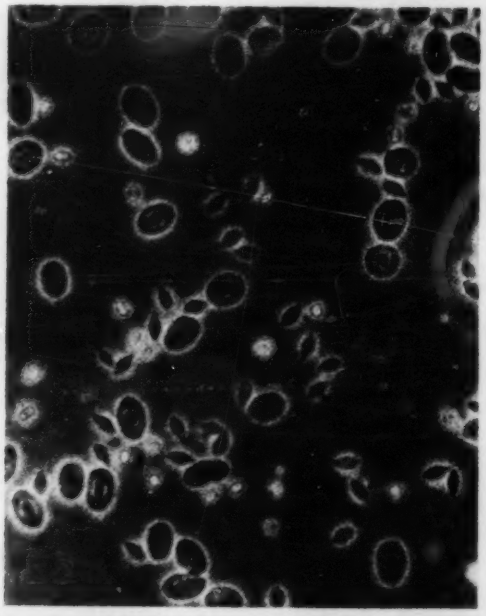


Figure 15A

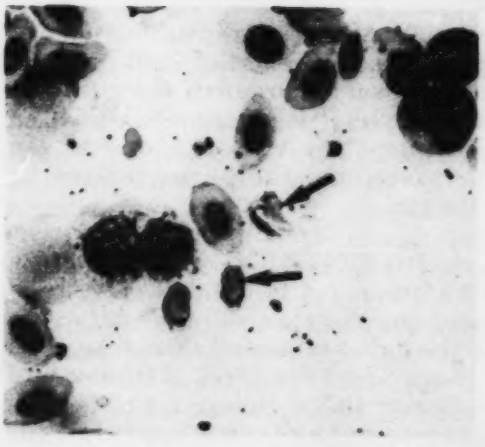


Figure 15B

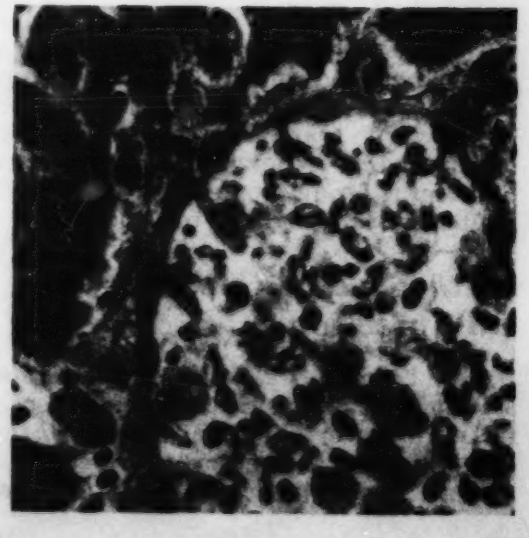


Figure 15C

Figure 15. Systemic diplomonad flagellate resembling Hexamita salmonis *(arrows) from chinook. A. Phase contrast wet mount of blood B. Liver imprint stained with Giemsa. C. Blood vessel in liver. H & E.*

MICROSCOPY. Wet mount preparations of the visceral organs reveals massive numbers of highly motile flagellates that are 10 X 5 µm (Fig. 15). The parasites are also readily detected in Leishman's Giemsa-stained imprints (Fig. 15), where they appear as dark-staining, oval bodies and two clear bands, representing the flagellar pocket, running through the length of the organism. Depending on the staining technique, two nuclei at the anterior end of the parasite may be visible.

Histological examination reveals massive numbers of the parasites in blood vessels in essentially all organs, with particularly high concentrations in the liver and the lamina propria of the lower intestine. The liver is edematous and shows diffuse infiltration of inflammatory cells resembling lymphoblasts and plasmablasts in the sinusoids. The renal interstitium is hyperplastic. Although many parasites are observed in blood vessels of the lamina propria and submucosa of the gut, the epithelium is usually intact.

DIAGNOSIS. The infection is identified by wet mount preparations or Giemsa-stained imprints of the blood or visceral organs. Because the parasite is highly motile, it may be easier to identify the parasites in wet mounts. In subclinical infections, the parasite may be confined to the gut.

CONTROL AND TREATMENT. Several drugs, most of which are added to the diet, have been recommended for the control of *H. salmonis* infections in the gut of salmonids (Yasutake et al. 1961; McElwain and Post 1968; Hoffman and Meyer 1974; Becker 1977). However, at this time, none of these compounds are approved for treatment of food fish in Canada.

Although we have determined that the disease is transmissible in sea water, we have yet to determine if the chinook contracted the infection in sea water, or were subclinically infected when they were transferred to netpens. An understanding of the source of the infection would be helpful for implementing effective control strategies or prophylactic treatments.

Ichthyobodo (=*Costia*) Gill Infections

The bodonid flagellate *Ichthyobodo necator* is a common ectoparasitic pathogen of fishes reared in fresh water (Becker 1977). *Ichthyobodo* infections have been observed on seawater-reared Atlantic salmon in Europe, and it was suspected that the fish acquired the infections in fresh water (Ellis and Wootten 1978; Roubal et al. 1987). However, *Ichthyobodo* has also been observed on strictly marine fishes, such as flatfishes and haddock (Cone and Wiles 1984; Bullock and Robertson 1982; Morrison and Cone 1986; Diamant 1987). Using cross infection studies, Urawa and Kusakari (1990) determined that *Ichthyobodo* sp. of Japanese flounder was a different species than *I. necator* of salmonids, and they showed that the parasite from fresh water can survive and proliferate on fish transferred to sea water. We have observed heavy *Ichthyobodo* infections associated with gill damage in pen-reared chinook from a few netpen sites in the Sechelt area of B.C. The parasite is transmitted directly from fish to fish. Multiplication is rapid, and untreated fish in confined environments can develop very heavy infections in a few days.

CLINICAL AND GROSS PATHOLOGY. Macroscopic changes in affected Pacific salmon have not been described. Heavily infected Atlantic salmon in Scotland were emaciated, anorexic, and swam near the surface (Ellis and Wootten 1978).

MICROSCOPY. Wet mount preparations of the gills and skin reveal numerous, motile parasites (Fig 13,16). The parasites are small (about 10-15 µm long), and free-swimming forms are oval with two pairs of uneven flagella. *Ichthyobodo* appears to swim with a jerky, spiral pattern. Attached forms are pyriform with obscure flagella.

Histological sections of affected gills reveal diffuse, epithelial hyperplasia of the gill epithelium and attached parasites are found on the epithelial surfaces (Fig. 16). Ellis and Wootten (1978) reported that heavily infected gills of pen-reared Atlantic salmon were infiltrated with melanomacrophages and there was an increase in goblet cells.

DIAGNOSIS. The infection is identified by observing numerous flagellate parasites with the morphology described above on the gills or skin in wet mounts. *Ichthyobodo* can also be identified on gill surfaces in histological sections, but wet mounts are preferred because parasites may become dislodged from the gills during processing of tissues, and the small flagellates are more easily identified when they are actively motile.

CONTROL AND TREATMENT. External treatments with formalin and malachite green have been used successfully to treat *Ichthyobodo* infections in fresh water (Hoffman and Meyer

1974; Becker 1977). However, these treatments would be difficult to apply in netpens and should be applied with great caution. For example, attempts at treating Atlantic salmon in netpens in New Brunswick with formalin at 1:10,000 caused heavy mortality within an hour (D. Speare, Atlantic Veterinary College, P.E.I., Canada, pers. comm.).

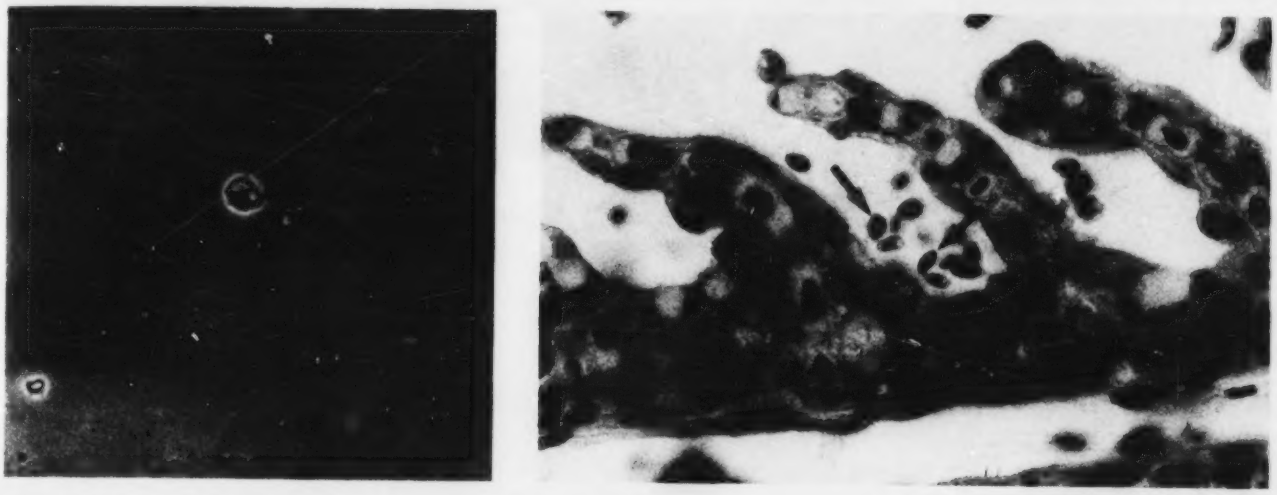


Figure 16. Ichthyobodo necator *(arrows) from the gill surface of a chinook salmon. A. Wet mount preparation, phase contrast. B. Tissue section. H & E.*

Myxosporeans

Myxosporean parasites (phylum Myxozoa, class Myxosporea) are common parasites of cold-blooded vertebrates, particularly fishes, and hundreds of species have been described (Lom and Noble 1984; Lom 1987). Most myxosporeans are relatively non-pathogenic. Histozoic species usually form small, confined white cysts with little associated tissue damage. However, when these cysts are numerous in vital organs, such as the gills or heart, they can cause disease. Furthermore, heavy infections of histozoic myxosporeans in the flesh may lower the market value of the affected fish. Pathogenic coelozoic species generally cause more diffuse infections without macroscopically visible cysts. Several species are recognized pathogens of salmonid fishes reared in fresh water (e.g., *Myxobolus cerebralis, Ceratomyxa shasta,* and the PKX myxosporean). Two myxosporeans, *Kudoa thyrsites* and *Parvicapsula* sp., have been associated with disease in seawater pen-reared salmon.

The life cycle of myxosporeans is complicated (Fig. 17). They contain several vegetative stages (trophozoites) and development in the fish culminates in the formation of multicellular spores. Spore morphology is the primary criterion used for identification of myxosporeans. The mode of transmission of most myxosporeans is poorly understood. At least for *Myxobolus* spp., it appears that development in an aquatic oligochaete is required to complete the life cycle (Wolf et al. 1986; El-Matbouli and Hoffman 1990; Kent et al. 1990b; Ruidisch et al. 1991; Yokoyama et al. 1991).

There are no commercially available drugs for treating myxosporean diseases of salmonids. However, Fumagillin DCH, a drug used for control of microsporidiosis in bees, has been shown to be efficacious for treating some myxosporean diseases (Molnár et al. 1987; Hedrick et al. 1988; Wishkovsky et al. 1990; Yokoyama et al. 1990; EL-Matbouli and Hoffmann 1991))

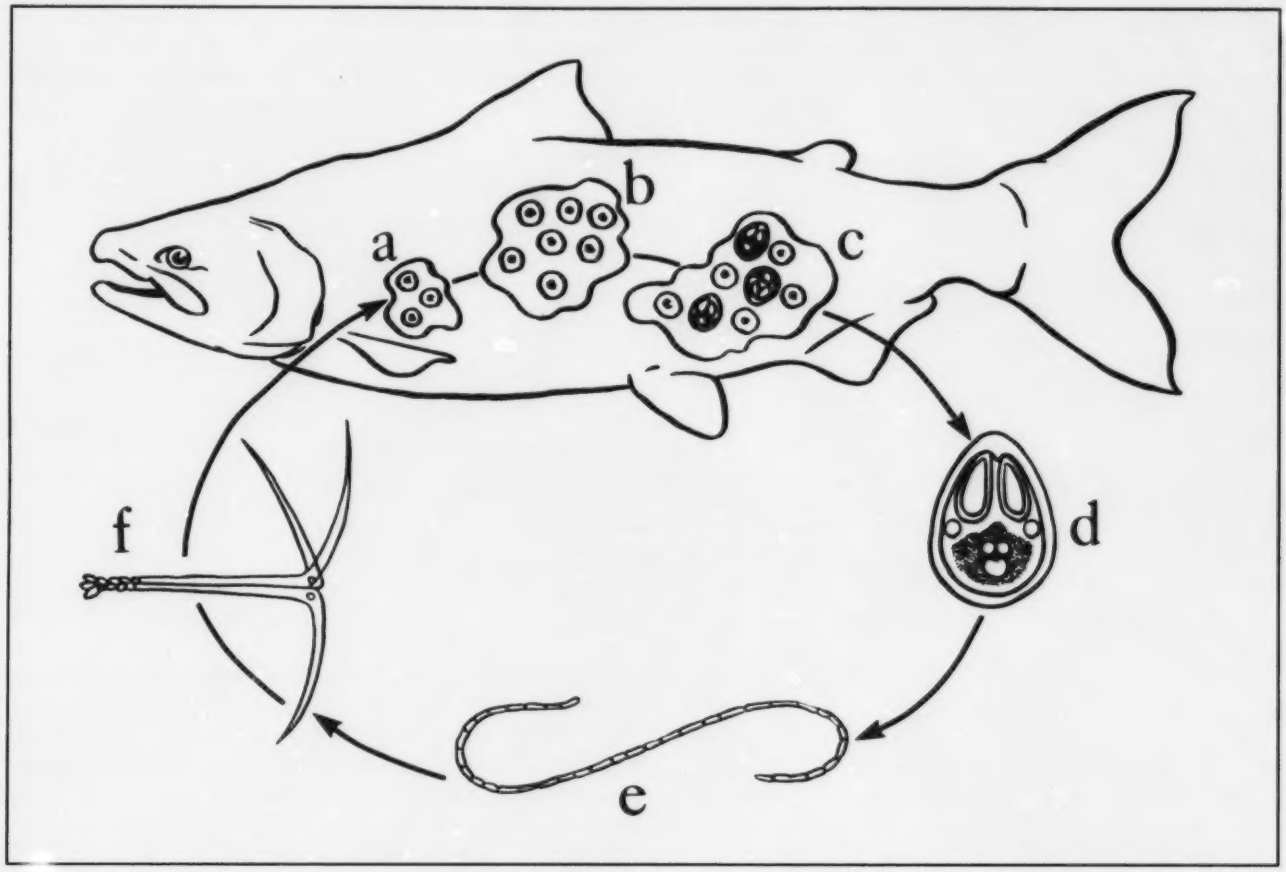


Figure 17. The life cycle and development of myxosporean parasites of fishes, exemplified by Myxobolus *spp.: a and b, vegetative development results in formation of a multinucleated plasmodium with many daughter cells; c, daughter cells develop into multicellular spores; d, spores are released from the fish to complete development and transmission of the parasite - spores are released after death for most histozoic species, whereas spores are released in feces or urine in coelozoic species; e, development of the parasite after release from the host is unknown for most myxosporean genera, but for* Myxobolus *spp. an aquatic oligochaete worm is apparently a required intermediate host in which the spores develop into actinosporean stages; f, actinosporean stage is released from the worm and infects the fish host to complete the life cycle.*

Parvicapsula sp.

Parvicapsula sp. infects the kidney of salmon reared in netpens, and in the early 1980s it was reported to be the cause of severe disease in pen-reared coho in Washington State (Hoffman 1984; Johnstone 1984). However, concurrent infections with *Renibacterium* and *Vibrio* often occurred, and the role of the parasite in the overall mortality was unclear.

Johnstone (1984) also observed *Parvicapsula* in chinook, Atlantic, and masou salmon, and cutthroat trout. A *Parvicapsula* sp. was observed in Pacific cod that were collected near pens containing infected coho. The parasite in Pacific cod appeared similar to the *Parvicapsula* of salmon, and Johnstone (1984) suggested that Pacific cod may be the reservoir for infections. The parasite has been detected in coho at one netpen site in B.C., and in wild sockeye salmon and coho on the west coast of British Columbia.

CLINICAL SIGNS AND GROSS PATHOLOGY. There are no pathognomonic clinical changes associated with parvicapsulosis. However, infected fish often are dark and lethargic, and exhibit kidney hypertrophy. *Parvicapsula* is a coelozoic myxosporean and macroscopically visible cysts containing the parasite are not observed.

MICROSCOPY. Histological sections of heavily infected kidneys show numerous trophozoites and

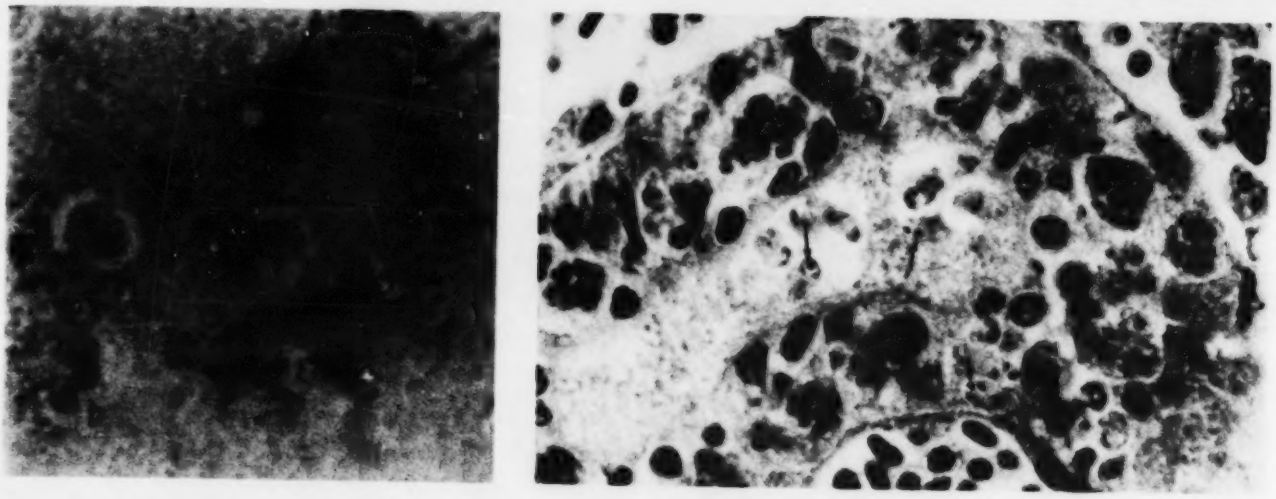


Figure 18. A. Gram stain of Parvicapsula *sp. spores. Arrows = polar capsules. B. Tissue section of coho kidney with* Parvicapsula *sp. infection. Developing parasites (P) cause necrosis and degeneration of the epithelium of renal tubules. Giemsa.*

developing spores in the epithelium and lumina of tubules. The epithelium is often necrotic and displaced by developing parasites (Fig. 18). Trophozoites also occur in the blood and kidney interstitium and may cause interstitial nephritis. Spores are elongated, 7-10 X 3- 5 µm (measured from tissue sections) and have two tiny polar capsules at the anterior end (Figs. 13, 18).

DIAGNOSIS. Parvicapsula spores are very small and identification of this parasite is usually based on histological examinations. Positive identification is based on observation of the spore, which are readily visible in histological sections stained with Giemsa. Spores can also be detected in Gram stained kidney smears (Fig. 18).

CONTROL AND TREATMENT. No drugs are available to treat the infection. Pacific cod may be a reservoirs for infection, but control of these fish is impractical in the netpen situation.

Kudoa thyrsites

Myxosporeans of the genus *Kudoa* and related genera infect the muscle of many marine fishes, and heavy infections can cause unsightly white cysts or soft texture in fillets (Kabata and Whitaker 1981; Patashnik et al. 1982). These parasites are therefore of concern because they can lower the market value of the infected fish, although they seldom cause morbidity. *Kudoa thyrsites* infects the muscle of pen-reared Atlantic salmon (Harrell and Scott 1985; Whitaker and Kent 1992). This myxosporean usually infects Pacific hake, and Atlantic salmon may be abnormal hosts for the parasite. In one instance, Harrell and Scott (1985) attributed mortalities in Atlantic salmon smolts to this parasite. More importantly, heavy infections associated with soft flesh and the unsightly white patches have been observed in several shipments of pen-reared Atlantic salmon from B.C. and Washington (Fig. 20). Based on observations by processors, the prevalence of infection in pen-reared Atlantic salmon ranges from 2-25%. Often the condition is unnoticed until fillets are smoked. In the investigation of *K. thyrsites* infections in Pacific hake, it was found that the flesh softening was caused by a proteolytic enzyme produced by the parasite (Tsuyuki et al. 1982). This enzyme remains active at temperatures below 70 °C. Therefore, tissue breakdown will continue through most smoking processes, which are normally conducted at 50 °C or less.

CLINICAL SIGNS AND GROSS PATHOLOGY. Heavily infected fish exhibit white streaks or patches in the muscle and the texture of the muscle is soft (Fig 20).

MICROSCOPY. Wet mount preparations of infected fillets reveal stellate myxosporean spores with four valves and four polar capsules converging at one end (Figs. 13, 19). The spores are approximately 13 µm from tip to tip.

Developing parasites occupy muscle fibers forming large "pseudocyts" filled with developing spores. Although the parasite elicits little tissue reaction, heavy infections are associated with extensive damage to surrounding muscle fibers. Infections have also been observed in the cardiac

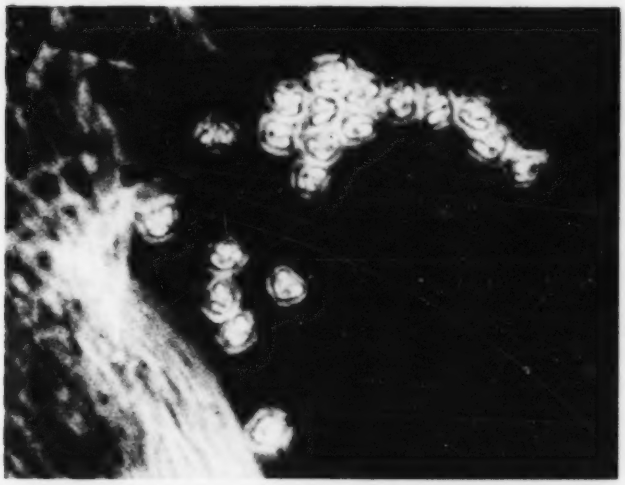


Figure 19. Wet mount of Kudoa thyrsites. *Note stellate spores with four polar capsules.*

muscle of wild Pacific salmon (Kabata and Whitaker 1989) and Atlantic salmon (Brackett et al. 1991).

DIAGNOSIS. Diagnosis is based on the observation of the characteristic stellate spores of the parasite (Fig. 13,19). The spores are best detected by microscopic examination of fluid collected from the freshly cut surface of a fillet. The parasite shows up well in Giemsa-stained histological sections. Detection of the parasite in fish in the round presents a problem that is, as yet, unresolved.

TREATMENT AND CONTROL. No drugs are available for treating *Kudoa* infections. Fish become infected in sea water, so it would be very difficult to eliminate exposure to infections.

Figure 20A

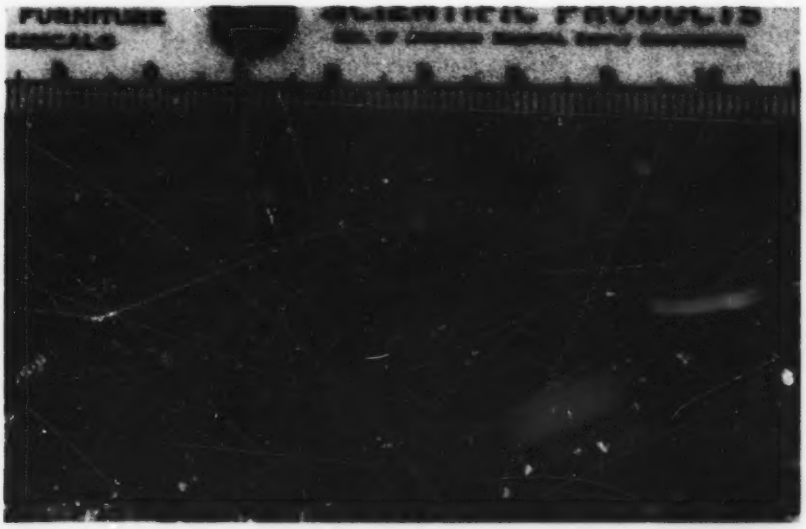


Figure 20B

Figure 20C

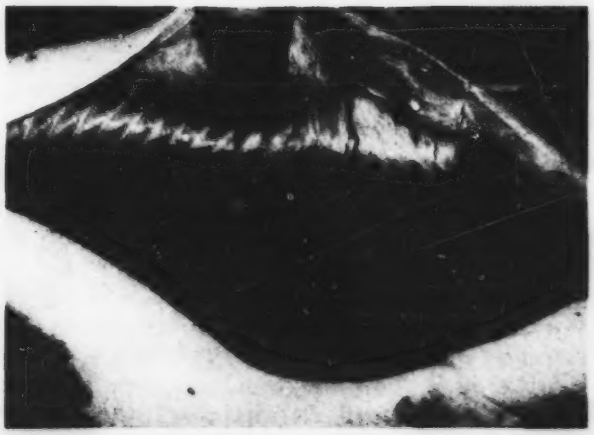


Figure 20D

Figure 20E

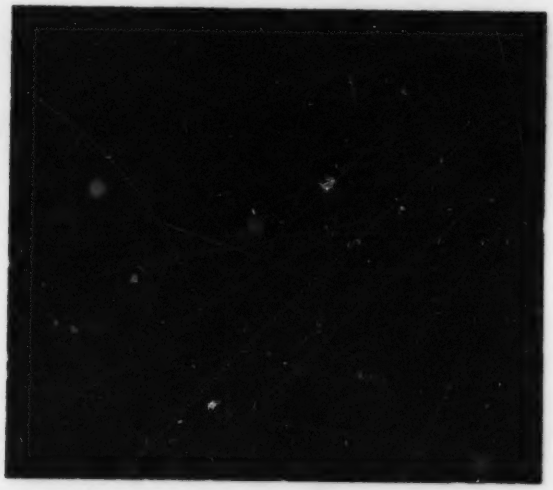


Figure 20F

Figure 20. Protozoan parasites from pen-reared salmon A. Rainbow trout with Paramoeba *gill infection. Note white areas in gill filaments. (Courtesy of C.K. Foster). B. Multifocal white patches in smoked Atlantic salmon fillet with* Kudoa thyrsites *infection. C, D. Chinook with systemic hexamitosis. C. The liver (L) is enlarged, mottled and hemorrhagic, and large blood clots (B) occur in the visceral cavity. D. The abdomen is swollen and filled with a serosanguinous ascites (S). E. Chinook gill with* Loma salmonae *infection. Arrow = xenomas. F.* Loma salmonae *in Gram-stained gill imprint from chinook. Some spores are strongly Gram-positive, while others are unstained (arrows). Bar = 10 µm. G.* Enterocytozoon salmonis *in Gram-stained kidney imprint from chinook. Spores are strongly Gram-positive and some occur within nuclear remnants (arrow). Bar = 10 µm.*

Figure 20G

Microsporidians

Microsporidians (phylum Microspora) are common protozoan parasites in the aquatic environment and many cause disease in fishes and invertebrates (Canning and Lom 1986). Like myxosporeans, they undergo complex development that culminates in a spore with a coiled polar tube (Fig. 21, pg. 44). In contrast to myxosporeans, microsporidian spores contain no polar capsules and the polar tube lies free in the sporoplasm. Additionally, microsporidians contain no mitochondria and all are obligate intracellular parasites. Two microsporidians infect seawater-reared salmonids in the Pacific Northwest; *Loma salmonae* infects the gills and visceral organs of coho and chinook, and *Enterocytozoon salmonis* infects the nuclei of blood forming cells in chinook.

Loma salmonae

Loma (formerly *Pleistophora*) *salmonae* infects the gills and other vascularized tissues of salmonids reared in fresh water (Putz et al. 1965; Putz and McLauglin 1970; Hauk 1984; Morrison and Sprague 1981, 1983). Severe gill infections have been reported in rainbow trout, steelhead trout and kokanee salmon (Wales and Wolf 1955), and Hauck (1984) observed high mortality in chinook due to systemic infections by a *Loma* sp. (presumably *L. salmonae*). Infections in coho in fresh water are usually mild and cause little disease (Magor 1987).

Infections can persist after fish are transferred to sea water, and the associated lesions in the gills can become severe in pen-reared salmon (Kent et al. 1989; Speare et al. 1989). Although the gills are the primary site of

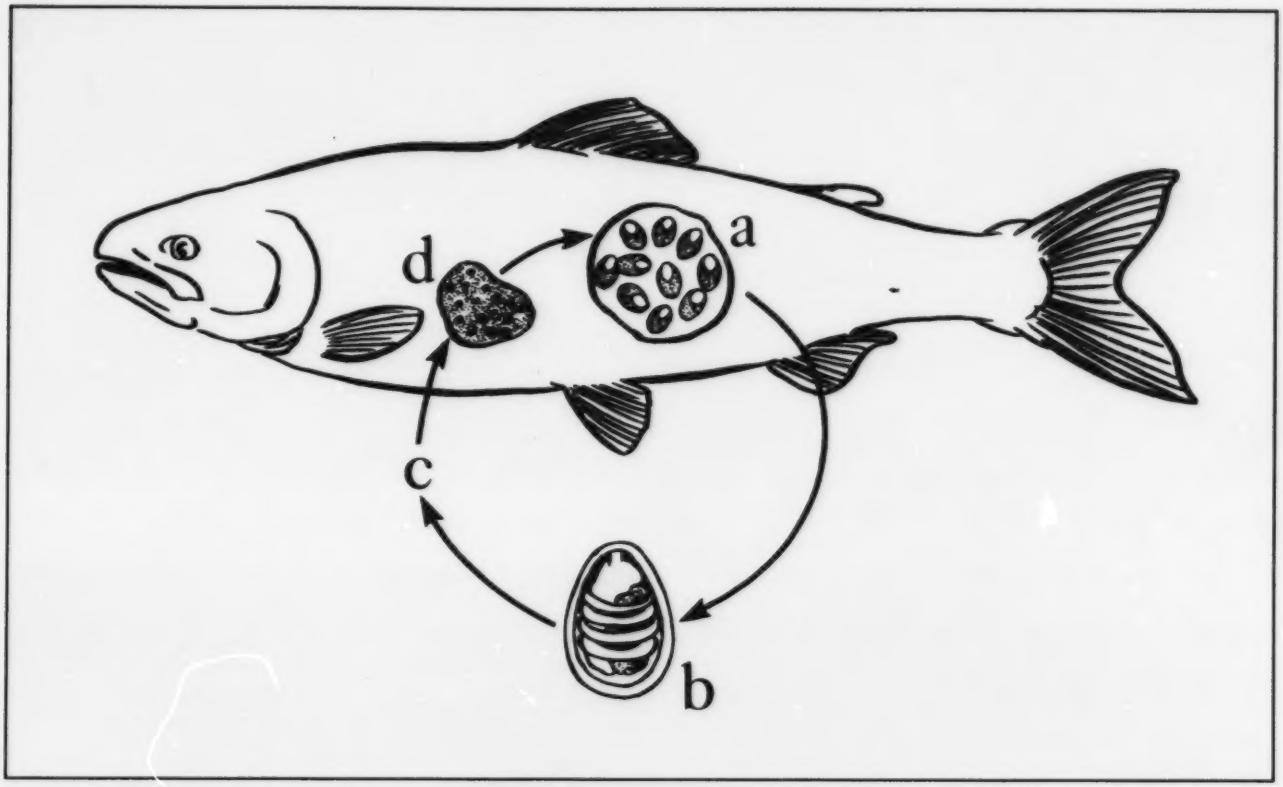


Figure 21. Generalized life cycle of microsporidians: a, xenoma with mature spores; b. spores released from host after xenoma ruptures (possibly after death of the host) - typical microsporidian spore with coiled polar tube in the peripheral cytoplasm; c, fish are apparently infected directly by ingesting spores; d, a sporoplasm is released from the spore and infects a host cell - the parasite undergoes intracellular vegetative development (merogony and sporogony), culminating in the formation of spores in xenomas.

infection, parasites and associated lesions can occur in the heart, spleen, kidney and pseudobranchs.

CLINICAL SIGNS AND GROSS PATHOLOGY. Affected fish do not exhibit any distinguishing clinical signs, but fish with severely damaged gills may exhibit respiratory distress. Close examination of heavily infected fish may reveal small white cysts in the gills (Fig. 20). Infected gills may also appear lumpy. Systemic infections in chinook may cause enlargement of the spleen and kidney.

MICROSCOPY. Loma salmonae spores can be detected in wet mount preparations of heavily infected gills. The spores are bean-shaped, about 5.5 X 3 µm, and have a posterior vacuole (Fig. 13,22). Gram-stained smears of heavily infected tissue reveal the Gram-positive spores of *L. salmonae* (Fig 20). Histological examination reveals the cyst-like xenomas formed by the parasite (Fig. 22). Xenomas that develop in the secondary lamellae of the gills cause relatively little damage and inflammation, whereas those that form deep within the blood vessels of primary lamellae can cause severe inflammation and tissue destruction (Fig. 22). In such cases, microsporidian spores are dispersed throughout inflammatory lesions and individual spores are found within phagocytic cells. Similar inflammatory lesions caused by ruptured xenomas are found in the spleen and kidney, especially in chinook. Release of spores from xenomas into surrounding tissue is believed to be a factor contributing to the inflammatory response (Hauck 1984).

DIAGNOSIS. Loma salmonae infections are diagnosed by detecting the characteristic spores. The spores can be demonstrated in wet mounts or Gram-stained tissue smears of gills when infections are heavy. However, the gills may show severe pathological changes with relatively few spores, and histology should be conducted on suspect tissues because the parasites are difficult

to detect in wet mounts when the xenomas have ruptured. Furthermore, tissue sections will reveal the characteristic inflammatory lesions in the gill primary lamellae and visceral organs.

Figure 22. Microsporidians from chinook salmon. A. Wet mount of Loma salmonae *spores. B, C. Tissue sections of coho gills with* L. salmonae *infection. H & E. B. Intact xenoma with little tissue reaction. C. Chronic inflammation in the primary lamella due to* L. salmonae *infection. (Courtesy of Aquaculture). D.* Enterocytozoon salmonis *(arrows) in the nuclei of plasmablasts of a chinook salmon. H & E.*

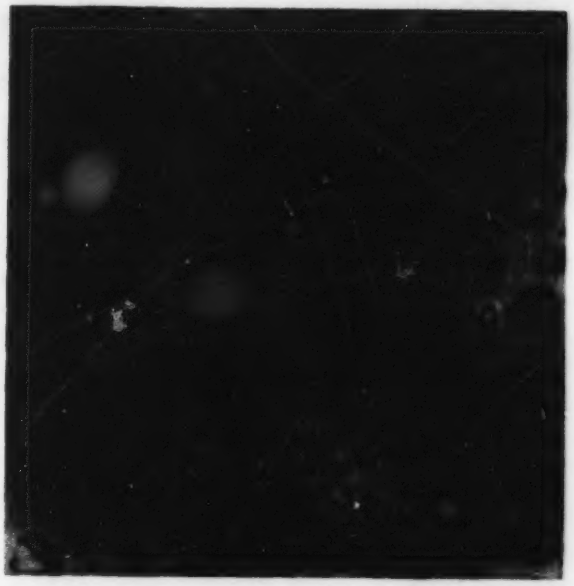


Figure 22A

Figure 22D

Figure 22B

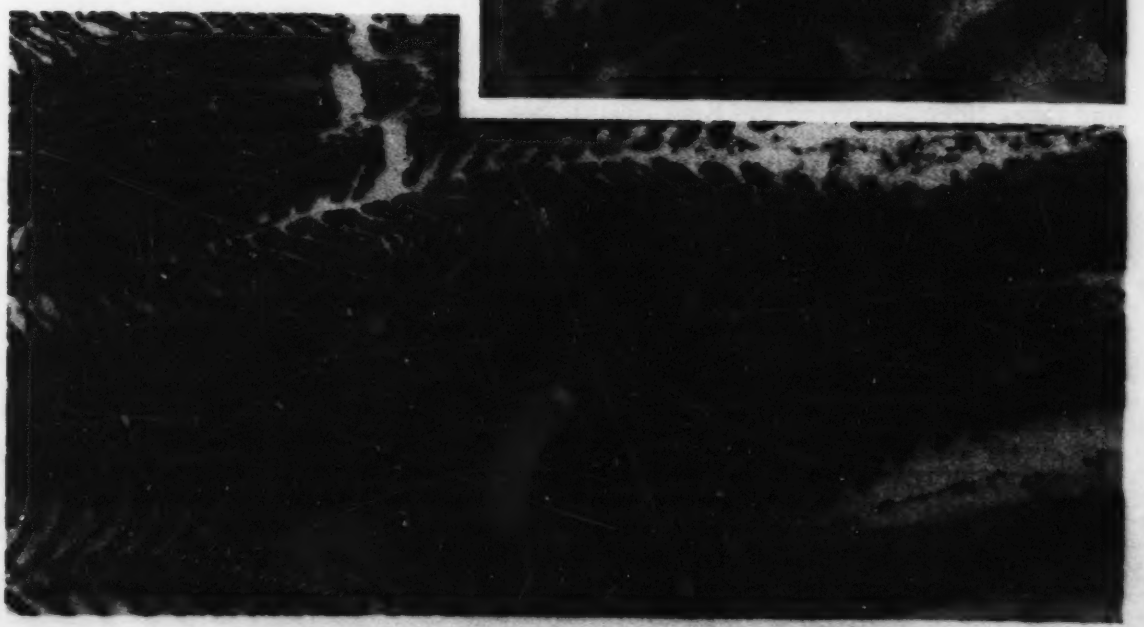


Figure 22C

TREATMENT AND CONTROL. Under experimental conditions, Fumagillin DCH has been used to treat microsporidiosis in eels (Kano and Fukui 1982) and infections of the microsporidium *Enterocytozoon salmonis* in chinook (Hedrick et al. 1991), but no drugs for treatment of *L. salmonae* are commercially available. However, the disease could be controlled by avoiding infections. Infections originate in fresh water and persist in fish after they are transferred to netpens. It is not known if the parasite is transmitted in sea water. Ensuring that fish are free of infection during their freshwater development may prevent introduction of the parasite to netpens.

Enterocytozoon salmonis

Enterocytozoon salmonis is an unusual microsporidium that infects the nuclei of hemoblasts, particularly lymphoblasts or plasmablasts, in salmonid fishes (Chilmonczyk et al. 1991). This microsporidium was first observed in pen-reared chinook in Washington State, where it was associated with anemia (Elston et al. 1987). The parasite has also been reported in freshwater-reared chinook, kokanee, and steelhead trout in Washington (Morrison et al. 1989), California (Hedrick et al. 1990; 1991) and Idaho (MacConnel et al. 1991). Although *E. salmonis* has not been detected in freshwater-reared salmon in B.C., it has been observed in chinook at several seawater netpen sites in the Province. The infection in B.C. is often associated with a concurrent neoplastic disease involving massive lymphoproliferation, known as plasmacytoid leukemia (PL) (Kent et al. 1990). Laboratory transmission studies indicate that *E. salmonis* is not the primary cause of PL (Kent and Dawe 1990; Newbound and Kent 1991a). See pages 59 - 62 for further details on the relationship of *E. salmonis* with PL.

CLINICAL SIGNS AND GROSS PATHOLOGY. Heavily infected fish are anemic, with a packed blood cell volume as low as 5%. Typical of severe anemia in fish, affected salmon exhibit prominent pallor of the gills. See page 59 - 61 for description when infections are associated with PL.

MICROSCOPY. This microsporidium is extremely small and is identified by careful examination of nuclei of hemoblasts in histological sections (Fig. 22). Some researchers have also used Giemsa-stained impression smears to identify the parasite. In tissue sections stained with hematoxylin and eosin, the parasites appear as eosinophilic spherical bodies (2-4 µm) in host cell nuclei, surrounded by a rim of basophilic host cell chromatin. In Giemsa imprints, the parasites appear as clear spheres within the host cell nuclei. The spores of microsporidians are Gram positive, and *E. salmonis* spores can be detected in Gram-stained smears of infected tissues (Fig. 20). In these preparations, the spores stain blue, have a characteristic bean shape, measure about 1 X 2 µm, and may exhibit a single vacuole. Electron microscopy is required to demonstrate more details of the morphology of spores and presporogonic stages (Fig. 23).

DIAGNOSIS. The infection can be diagnosed by detecting the characteristic spores in Gram-stained kidney or eye imprints. However, in some infections very few spores are found, and histology is required to detect the presporogonic forms within the nuclei of hemoblasts. Giemsa-stained imprints have been used by some for detecting these presporogonic forms, but because the parasites only appear as clear vacuoles in these preparations they could be easily confused with artifacts.

TREATMENT AND CONTROL. There is no commercially available drug for treating *E. salmonis* infections. However, Hedrick et al. (1991) controlled the infection in experimentally infected chinook with oral treatment of Fumagillin DCH at 1.0 mg drug/kg fish/day for 2 wk. To reduce the effects of the associated anemia, clinically affected fish should not be be handled or transported, and feed should be reduced to decrease oxygen demand.

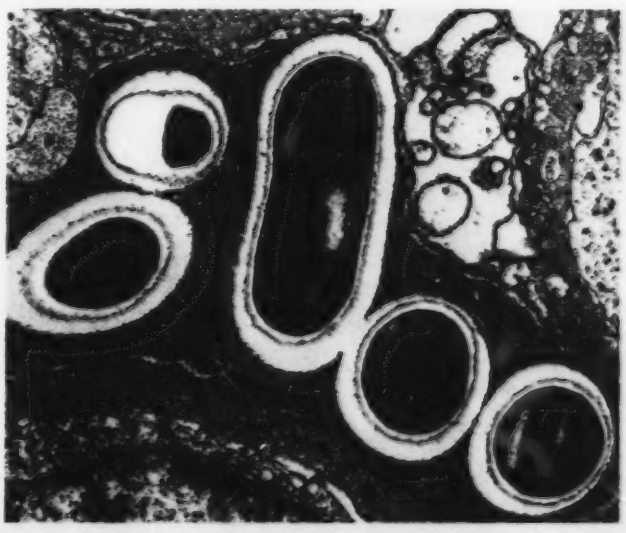


Figure 23. Electron micrograph of Enterocytozoon salmonis *spores in a host cell nucleus.*

HELMINTH PARASITES

By M. L. Kent and L. Margolis

Fishes are infected with a wide variety of parasitic worms, collectively referred to as helminth parasites. Although these parasites are very common in wild fishes, and occasionally infect cultured species, most often they do not cause severe disease. However, certain helminths can cause disease when infections are heavy or when they infect a critical organ. In addition, some helminth parasites of fishes can infect humans. The following are helminth parasites of importance in pen-reared salmon in the Pacific Northwest.

Cestodes (Tapeworms)

Fishes are infected by a wide variety of cestodes. Two life stages of cestodes are found in fish: adults infect the digestive tract and metacestodes (juveniles) are found in the internal organs and muscle, or occasionally other sites. The first intermediate hosts of tapeworms that infect fishes are usually arthropods (e.g., copepods).

Fishes are the second intermediate host for tapeworms in which a fish-eating mammal or bird, or another fish, are the definitive hosts. Fish usually acquire metacestode infections by eating infected arthropods. Metacestodes in fish tissues often cause an inflammatory response to the encysted or migrating parasite. The only reported significant metacestode disease of salmon reared in netpens in the Pacific Northwest is caused by *Gilquinia squali*, which infects the eyes of chinook.

Adult cestodes are often found in the

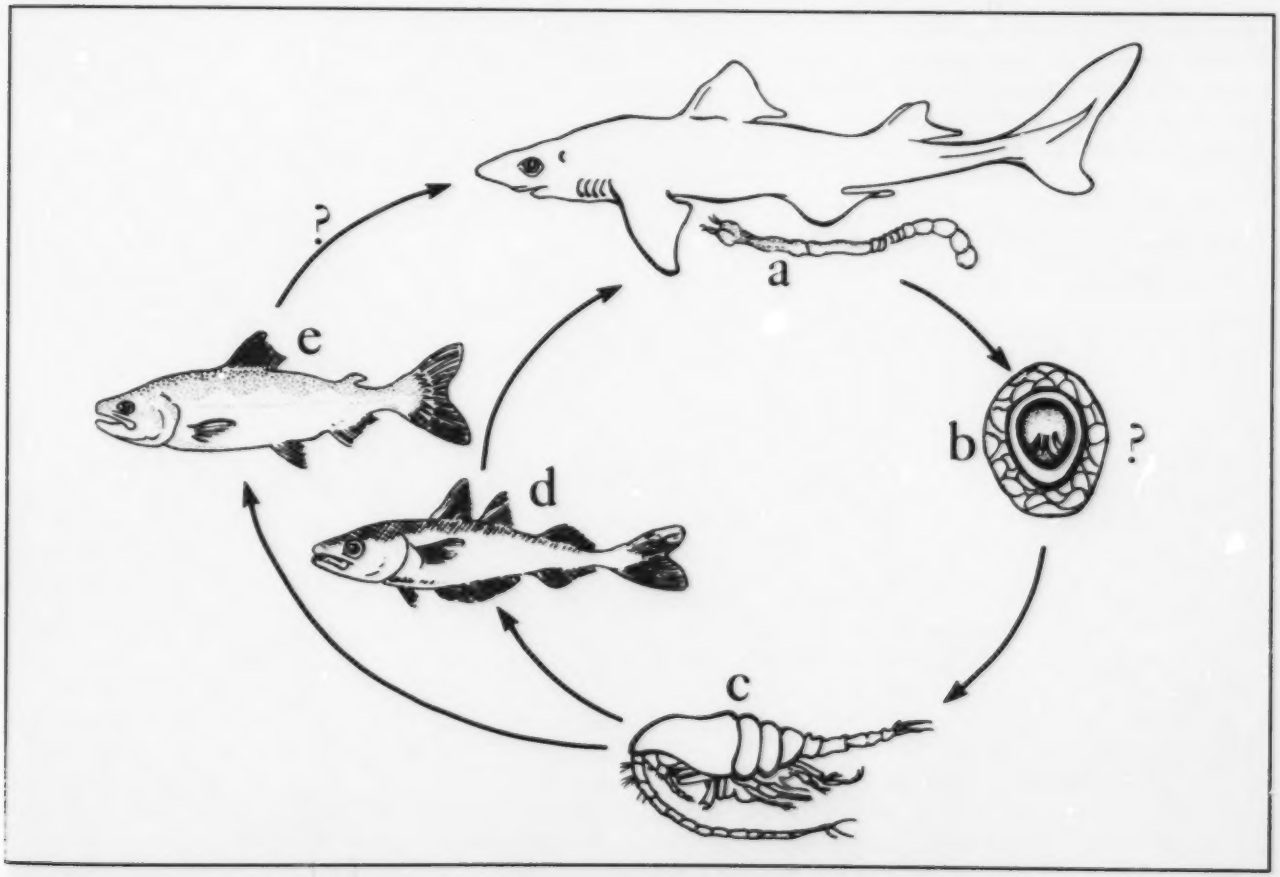


Figure 24. Life cycle of the trypanorhynch cestode Gilquinia squali*: a; adult worm in spiral valve of dogfish; b, coracidium develops within egg; c, the invertebrate first intermediate host (presumably a crustacean) is probably infected by eating an embryonated egg and the parasite becomes a procercoid in the hemocoele of the invertebrate; d,e, teleost fish (e.g., salmon, whiting) ingests invertebrate infected with the procercoid which migrates to the eye and becomes the plerocercoid (metacestode).*

intestinal tract of fishes. For example, *Eubothrium crassum* infects the intestine and pyloric caeca of salmon in sea water (Margolis 1982). The fish acquire the infection by ingesting first intermediate hosts (presumably copepods) infected with the procercoid stage, or possibly transport hosts infected with plerocercoids. The life cycle of this tapeworm has not been elucidated, but its freshwater counterpart, *E. salvelini*, in sockeye salmon in British Columbia uses copepods (*Cyclops* spp.) as its intermediate host. Procercoids that develop in *Cyclops* are directly infective for juvenile sockeye salmon (Boyce 1974). *Eubothrium crassum* occurs in wild caught salmon, and has been observed in brood stock chinook from netpens. Although infections can be intense, we know of no significant disease associated with the infections in salmon in the Pacific Northwest. However, poor growth and mortality has been associated with heavy *Eubothrium* sp. infections in pen-reared Atlantic salmon in Norway (Bristow and Berland 1991a,b). In addition, *Eubothrium salvelini* is known to affect survival, growth, and stamina, and to have other debilitating effects on juvenile sockeye (Boyce and Behrens-Yamada 1977; Boyce 1979; Boyce and Clarke 1983).

Gilquinia squali metacestodes in chinook

Eye infections by metacestodes of *Gilquinia squali* (order Trypanorhyncha) have been associated with mortality of young chinook at netpen sites in British Columbia (Kent et al. 1991b). The definitive hosts for trypanorhynch tapeworms are sharks and rays, and the definitive host for *G. squali* is the spiny dogfish (Fig. 24). The metacestode is common in the eyes of North Sea whiting (MacKenzie 1965, 1975). Kuitunen-Ekbaum (1933) reported this metacestode from the muscle of speckled sanddab from British Columbia coastal waters. Observations of the infection in pen-reared chinook is the first reported occurrence of *G. squali* metacestodes in a salmonid fish, which suggests that wild salmon are probably not a normal intermediate host for the worm.

The adult worms are prevalent in dogfish throughout B.C. waters, and gravid adult worms are commonly found in dogfish in the spring. Dogfish are frequently found in or around netpens at this time, thus providing infectious coracidia for the first intermediate host near the pens. Based on the few trypanorhynch cestode life cycles that have been investigated (Mudry and Dailey 1971; Overstreet 1978; Mattis 1986; Sakanari and Moser 1989), it is likely that a crustacean, which has yet to be identified, is the first intermediate host for *G. squali*. Chinook in netpens presumably acquire the infection by eating this crustacean.

Figure 25. Gilquinia squali *in chinook salmon. A. Chinook salmon with opaque lens associated with* G. squali *infection. B. Whole mount of* G. squali *mestacestode from the eye of chinook salmon. Note the four eversible, spiny tentacles that emerge from the apex of the scolex, which are characteristic for this group of tapeworms. Semichon's acetocarmine. (Courtesy of J. Aquat. Anim. Health).*

CLINICAL SIGNS AND GROSS PATHOLOGY. Moribund fish are lethargic and remain near the bottom of the pen. The fish are easily captured by hand by divers, suggesting that they might be blind. However, fish are not usually emaciated and contain food in their stomachs, which indicates that they continue to feed. The actual mechanism that causes death is unknown. The lens often appears normal, whereas the iris and vitreous humor may be white and opaque and occasionally exhibit hemorrhages. The lens in more severely affected eyes is also opaque, suggesting cataractous changes (Figure 25). In extreme cases, the globe is ruptured and the lens is extruded. However, many fish die with the globe of the eye still intact. The eye lesions are often bilateral.

MICROSCOPY. Dissection of affected eyes that are still intact reveals one or two metacestodes of *G. squali* in the vitreous humor (Fig. 25). The worms are white and are about the size and shape of rice grains when examined by the naked eye. Kent et al. (1991b) described the histopathological changes of *G. squali* infections in the eyes of chinook. Histological examination of infected eyes often reveals an inflammatory infiltrate in the vitreous humor surrounding the worm (Fig. 26). Separation of the sensory retina from the pigment epithelium causes moderate to severe folding of the retina, resulting in apparent retinal duplication. There is proliferation of the pigment cells, resulting in melanization of the choroid. The lens of infected eyes shows a spectrum of cataractous changes. Some severely affected eyes exhibit hemorrhages in the globe, congestion of the choroid, and extrusion of the lens. Panophthalmitis is prominent in eyes with a ruptured cornea. A tapeworm metacestode resembling *G. squali* was detected in the optic nerve of a chinook (R. Armstrong, B.C. Ministry of Agriculture and Fisheries, Abottsford, B.C., pers. comm.), which suggests that this may be the route of entry of the worm into the globe of the eye.

Figure 26. Gilquinia squali *(arrowhead) in the vitreous humor of the eye of a chinook salmon. Arrows indicate retinal folding and apparent duplication. H & E. (Courtesy of J. Aquat. Anim. Health).*

DIAGNOSIS. The infection is identified by detecting trypanorhynch metacestodes in the vitreous humor. Trypanorhynch cestodes are identified by the presence of four eversible, spiny tentacles that emerge from the apex of the scolex (Fig 25).

CONTROL AND TREATMENT. At present there is no known treatment for *Gilquinia* infections in fish. The disease, therefore, can only be controlled by preventing infections. The complete life cycle of the parasite is unknown, so precise recommendations for avoiding the infection are not available at this time. Furthermore, preventing transmission of the parasite from dogfish to salmon via the arthropod first intermediate host would be very difficult because of uncontrolled water movement into netpens and unrestricted movement of dogfish around netpens.

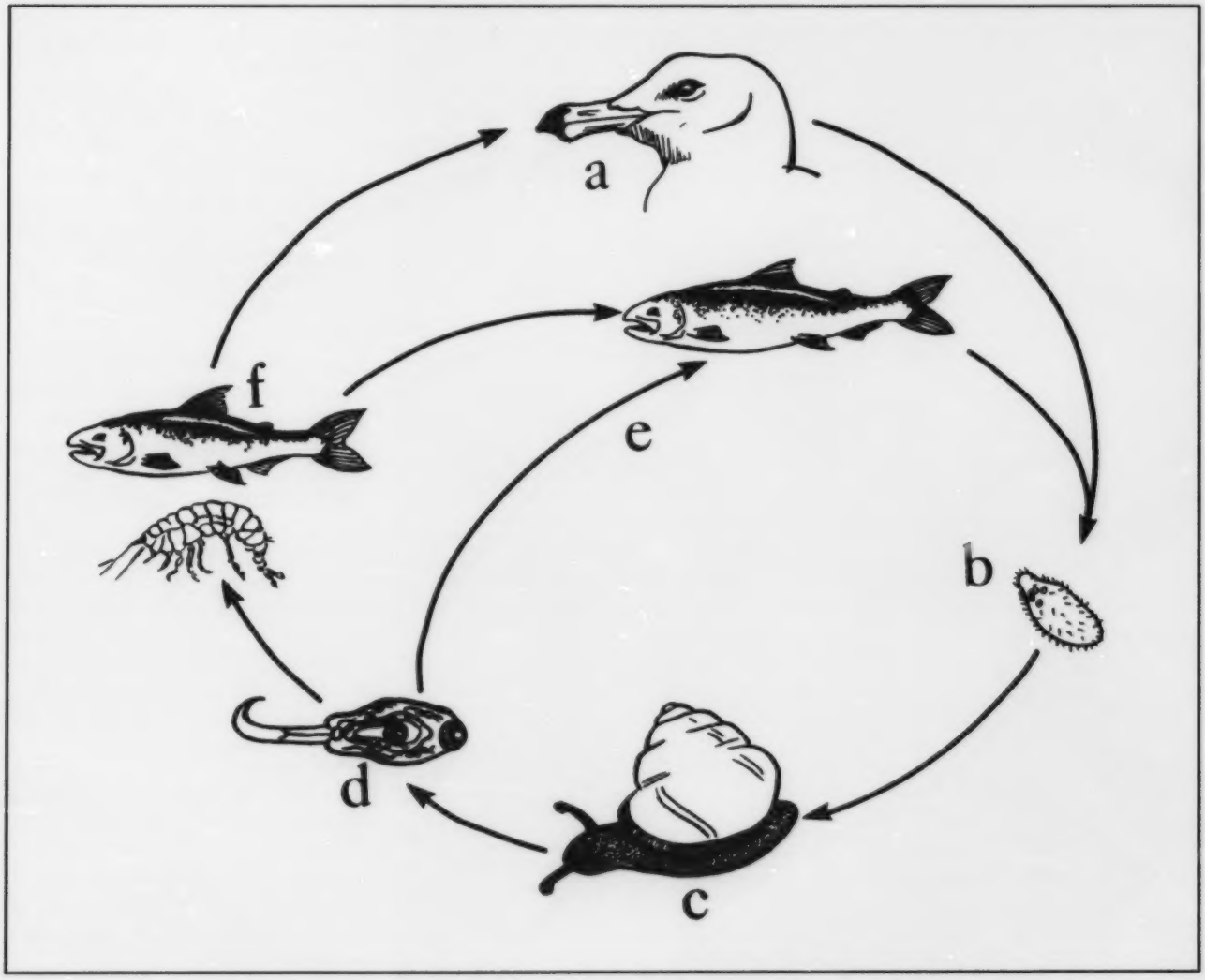


Figure 27. Generalized life cycles of digenean trematodes of fish: a, fish, bird, or mammal may be the definitive host for digeneans; b, adult worms release eggs and miracidia hatch from eggs in the external environment or within the next host; c, invertebrates, especially snails and bivalves, are first intermediate hosts in which the miracidium undergoes several developmental and proliferative stages culminating in numerous cercariae (d), that are shed from the host; e, certain digeneans (e.g., blood flukes) do not have a metacercarial stage and the cercariae infect the definitive host directly. f, fish or another invertebrate may be the second intermediate host, in which the cercariae encyst in tissues and becomes metacercariae. Definitive host eats second intermediate host and the parasite develops into an adult, usually in the gut.

Digenetic trematodes (flukes)

As with cestodes, fish can be intermediate hosts or definitive hosts for digenetic trematodes (Fig. 27). Molluscs, usually snails, are the first intermediate hosts of almost all digenetic trematodes. Cercariae emerge from molluscs and infect the second intermediate host, where they develop to metacercariae. Actively swimming cercariae penetrate the skin, migrate to the preferred site within the fish, encyst, and develop into a metacercaria. Metacercarial infections can occur essentially in any organ. Some of these infections, such as *Nanophyetus salmincola* (c.f. Baldwin et al. 1967) and *Diplostomum* spp. (c.f. Shariff et al. 1980), cause diseases in fish, whereas most metacercarial infections are relatively nonpathogenic. In addition, metacercarial infections of the skin or muscle can be important because they may reduce the aesthetic quality of the fish. For example, the black grub (*Neascus* sp.) has caused unsightly black lesions in the skin of coho in netpens.

Aquatic birds, mammals, or other fish serve as the definitive hosts for metacercariae of fishes. Except for blood flukes, most adult flukes of fish infect the alimentary tract and seldom cause significant tissue damage.

Neascus sp. (black grub) in coho

Black grubs (larval genus *Neascus*) are the metacercariae of certain species of the family Diplostomatidae. Black grubs infect a variety of fish in fresh water, including salmonids. Snails are the first intermediate hosts, and cercariae released from infected snails penetrate beneath the scales in the dermis of the fish host. Fish-eating birds serve as the definitive host. Mortality and morbidity have not been reported in infected salmon, but Lemly and Esch (1984) demonstrated that heavy infections could kill bluegill sunfish when water temperatures decline.

CLINICAL SIGNS AND GROSS PATHOLOGY. Infected fish exhibit numerous black, raised, nodules in the skin, which are about 1-2 mm in diameter (Fig 34, pg. 54).

MICROSCOPY. Careful examination of wet mount preparations of the black cysts reveals a metacercaria identified by the presence of two suckers (Fig. 28). Tissue sections of infected skin reveal encysted metacercariae surrounded by a thick fibrinous capsule (Fig 28). The periphery of the capsule contains numerous melanocytes.

DIAGNOSIS. Presumptive diagnosis can be obtained by the observation of small, multifocal, slightly raised black spots in the skin. Confirmation is obtained by observing metacercariae in the cysts in wet mount preparations or histological sections.

CONTROL AND TREATMENT. Fish become infected in fresh water by exposure to surface water containing infected snails. Disinfection of influent water or using ground water should eliminate or greatly reduce the infection. Based on reports from one fish farm, removing fish from freshwater hatcheries and

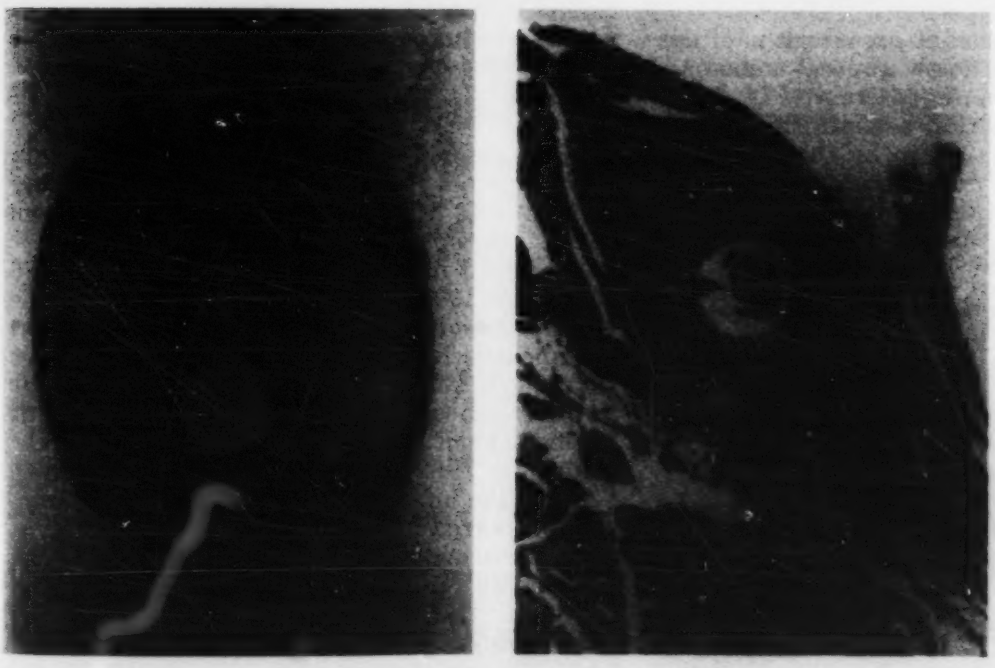


Figure 28. Black grub (Neascus sp.) metaceraria.
A. Parasite excised from a cyst. Note two suckers, which are characteristic of digeneans. (Courtesy of G.L. Hoffman).
B. Black grub (arrow) encysted in the dermis of coho.

introducing fish to netpens before June or July may reduce the intensity and prevalence of the infection.

Monogenean Flatworms

Monogenean flatworms, often incorrectly called monogenetic trematodes, are common parasites of the skin, fins, and gills of fishes. Several species cause disease in captive fishes. At least one monogenean, *Gyrodacytlus salaris,* can apparently cause mortality in wild Atlantic salmon (Johnsen and Jensen 1991). Unlike digenetic trematodes, monogeneans have a direct life cycle, and some are viviparous. Therefore, they can rapidly proliferate and spread in the confined environment of aquaculture systems. The only monogenean that we have detected in seawater-reared salmon is *Laminiscus strelkowi* (Fig. 29). Typical of its family, it is a small worm, approximately 0.5 mm in length. This parasite is frequently found on the gills of salmonids reared in netpens, and we have seen heavy infections in Atlantic and coho salmon. Very heavy infections have also been observed on the gills of pen-reared cutthroat trout (L.W. Harrell, Nat. Mar. Fish. Serv., Manchester, Washington, pers. comm.). The precise role of the parasite in disease in pen-reared salmon is unclear.

CLINICAL SIGNS AND GROSS PATHOLOGY. Fish with heavy monogenean infections of the gills are often lethargic, exhibit labored respiration, and accumulate near the surface.

MICROSCOPY. Wet mount preparations of the gills of heavily infected fish will reveal numerous, actively moving monogeneans (Fig. 29). Histological examination reveals the worms attached to the gill surface by the posterior attachment organ, the opisthaptor. Examination of moderately infected coho revealed no significant histological changes in the gills.

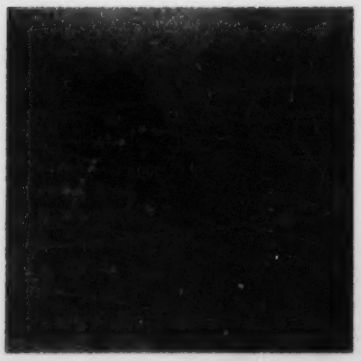


Figure 29. Monogenean trematode Laminiscus strelkowi *from the gills of pink salmon. Arrow= opisthaptor, used to attach to host tissue.*

DIAGNOSIS. Monogenean infections are diagnosed by observing the parasites in wet mount preparations of the gills or skin.

CONTROL AND TREATMENT. Monogenean infections are controlled with external therapeutic agents, such as formalin and organophosphates. However, both of these are not legal for treating food fishes in Canada (see pages 3 - 6).

Nematodes

Nematodes (roundworms) are common parasites of fishes, and occasionally infect pen-reared salmon. As with cestodes and digenetic trematodes, fish can be either definitive or intermediate hosts for nematodes. Crustaceans and, less frequently, other invertebrates are the first intermediate hosts for nematodes that infect fish. One nematode, *Philonema agubernaculum,* causes severe visceral infections in salmon reared in netpens in freshwater lakes. It is possible that these infections could persist in fish if they were transferred to seawater. However, diseases caused by nematodes have not been reported in seawater-reared salmon in the Pacific Northwest. The only nematode that we have detected in seawater-reared salmon is *Hysterothylacium* sp. (family Anisakidae). This is an apparently nonpathogenic nematode that was found in the digestive tract. However, we include a discussion of another anisakine nematode, *Anisakis* sp., which can infect humans. Larvae of this worm are common in wild-caught salmon and have the potential to infect pen-reared salmon.

Anisakine nematodes

Larvae of *Anisakis* spp., and the closely related genus *Pseudoterranova*, are commonly found in wild fishes. These worms are of concern because they can infect humans who eat raw or undercooked fish (Margolis 1977; Deardorff and Overstreet 1991). The general life cycle of these worms involves crustaceans as the first intermediate host, fish as second intermediate or transport hosts, and marine mammals as definitive hosts (Fig. 30). Most wild caught Pacific salmon are infected with *Anisakis* larvae (Margolis 1982; Deardorff and Throm 1988), whereas the infection has not been reported from salmon reared in netpens (Deardorff and Kent 1989; Bristow and Berland 1991a). However, farmed fish could become infected by feeding on infected fish (e.g., Pacific herring) or invertebrates that frequent netpens.

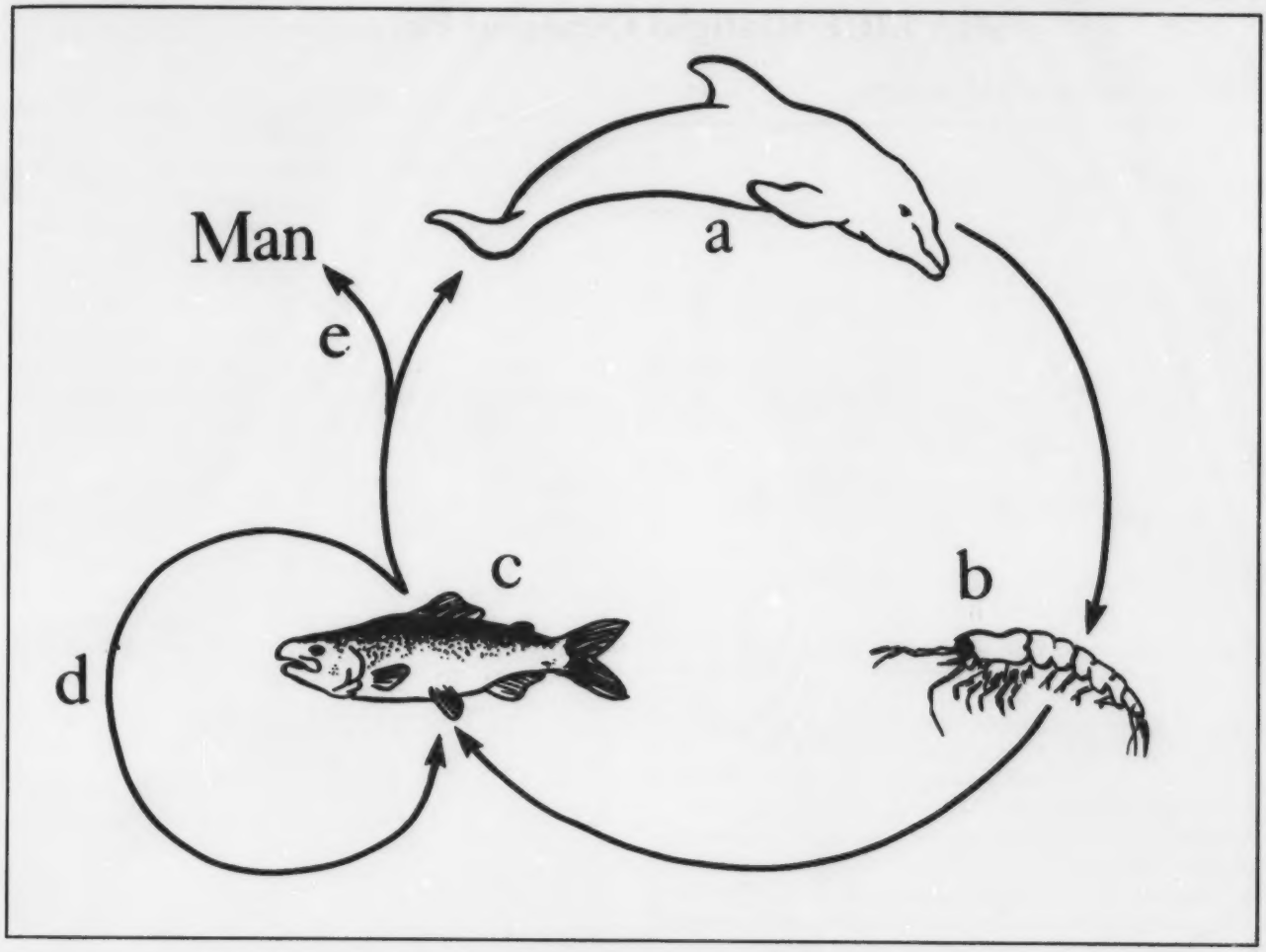


Figure 30. Life cycle of anisakine nematodes: a, marine mammals eats fish infected with third stage larvae (L3) - larvae develop into fourth stage larvae, then adults, in the gut of the mammal - eggs are shed from mammals, hatch and second stage larvae (L2) eaten by crustaceans (intermediate host); b, L2 develops into L3 in crustacean; c; fish or invertebrate (e.g., squid) are paratenic (transport) hosts; d; L3 can be transmitted from fish to fish, squid to fish, or vice versa; e, man becomes infected after eating undercooked or raw fish - man is a dead end host in which the worms remain as L3 larvae or molt into L4 larvae.

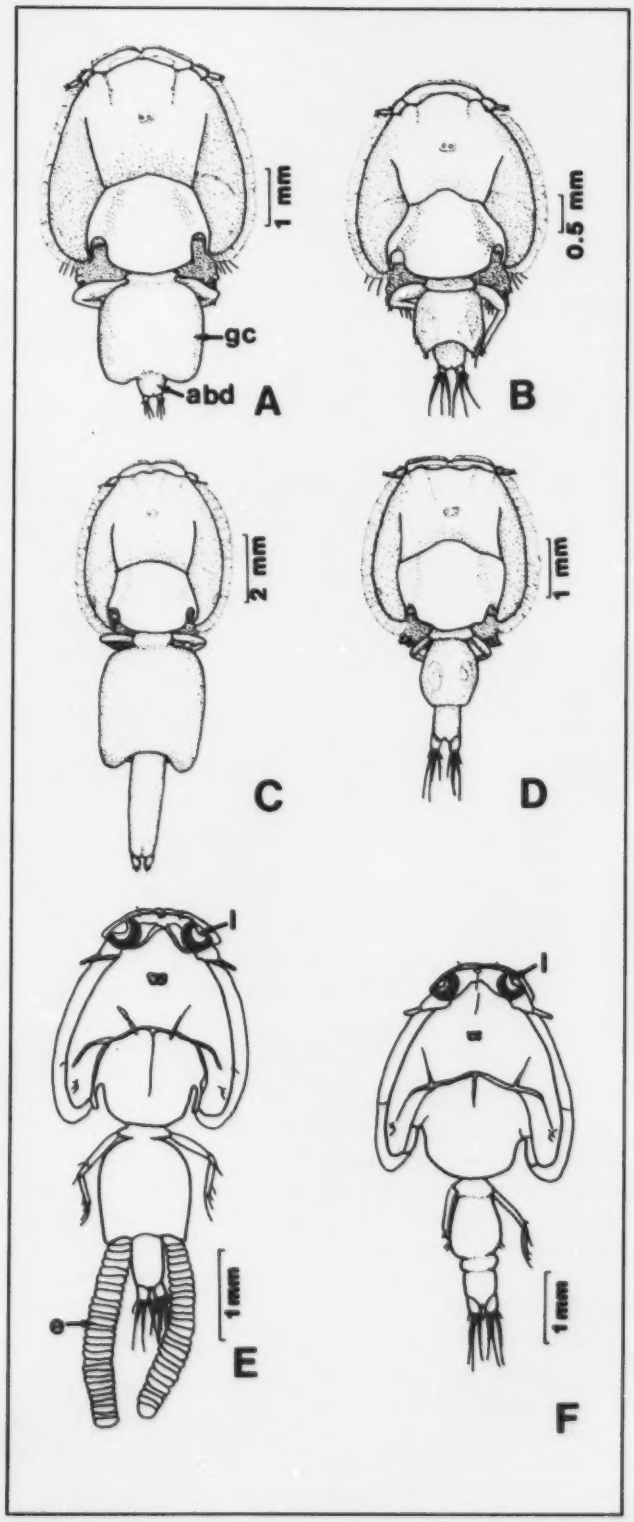
CLINICAL SIGNS AND GROSS PATHOLOGY. *Anisakis* larvae are relatively nonpathogenic to salmon and morbidity has not been associated with the infection. Dissection of infected fish reveals encysted worms in the liver, mesenteries and flesh (Fig. 34, pg. 55).

DIAGNOSIS. Diagnosis is based on the identification of the worm. Presumptive diagnosis is based on the observation of encapsulated nematodes in the viscera or body musculature. The worms are whitish and cylindrical, and usually range in length from 17 to 34 mm (Fig. 34). Confirmation of the worm's identity requires microscopical examination of cleared or dissected worms for certain pathognomonic anatomical features (Olson et al. 1983).

CONTROL AND TREATMENT. The apparent absence of *Anisakis* larvae in farmed salmon is probably due to the fact that most of the diet of the salmon consists of manufactured feeds. Some fish farmers have used fresh Pacific herring to supplement the diet of their fish. This practice should be avoided because herring are often heavily infected with the worm, which can be transmitted from prey to predator fish. However, frozen herring could be used because freezing fish for at least 24 h at -20 °C kills the worm.

SEA LICE (Caligid Copepod Parasites)

By S. C. Johnson and M. L. Kent



In the Pacific Northwest, two species of sea lice commonly infect and cause disease in sea-farmed salmonids: *Lepeophtheirus salmonis* (the salmon louse) and *Caligus clemensi* (Fig. 31). A third species, *Lepeophtheirus cuneifer*, occasionally infects salmonids but is not considered to be economically important (Johnson and Albright 1991a) (Fig. 31, 32).

Lepeophtheirus salmonis is essentially limited to salmonids and has been reported from all anadromous or seawater-reared salmonid species in the Pacific Northwest. *Caligus clemensi* and *L. cuneifer* occur naturally on a wide variety of hosts, mainly non-salmonids. Known salmonid hosts for *C. clemensi* include: pink, chum, chinook, coho, sockeye, and Atlantic salmon, and rainbow or steelhead trout. Common non-salmonid hosts of *C. clemensi* include: Pacific herring, threespine stickleback, copper rockfish, walleye pollock, spotted ratfish, and greenling. Known salmonid hosts for *L. cuneifer* include Atlantic salmon and rainbow or steelhead trout. Known common non-salmonid hosts of *L. cuneifer* include big skate and greenling.

All species and stages of salmonids reared in sea water in the Pacific Northwest are susceptible to sea lice infection. In British Columbia, Atlantic salmon and rainbow trout are generally more heavily infected with sea lice than chinook or coho when raised at the same site. Experimental transmission studies with *L. salmonis* using Atlantic, chinook and coho salmon showed that Atlantic salmon were the most susceptible to the infections, while coho was the most resistant species (Johnson 1991). The relationship of the number of lice to the severity of disease is dependent on 1) the size and age of the fish, 2) the general state of health of the fish, and 3) the species and developmental stages of sea lice present.

All species of sea lice that have been studied so far have a direct life cycle with ten developmental stages (Fig. 33). Fertile eggs are carried by females, and when they hatch they release a free-living planktonic nauplius stage. This is followed by another nauplius stage, a free-

Figure 31. Adult sea lice from salmonid fishes.
A, Lepeophtheirus cuneifer *female; B,* L. cuneifer *male; C,* L. salmonis *female; D,* L. salmonis *male; E,* Caligus clemensi *female; F,* C. clemensi *male. A and B redrawn from Kabata (1974); C and D redrawn from Johnson and Albright (1991b); E and F redrawn from Parker and Margolis (1964).*

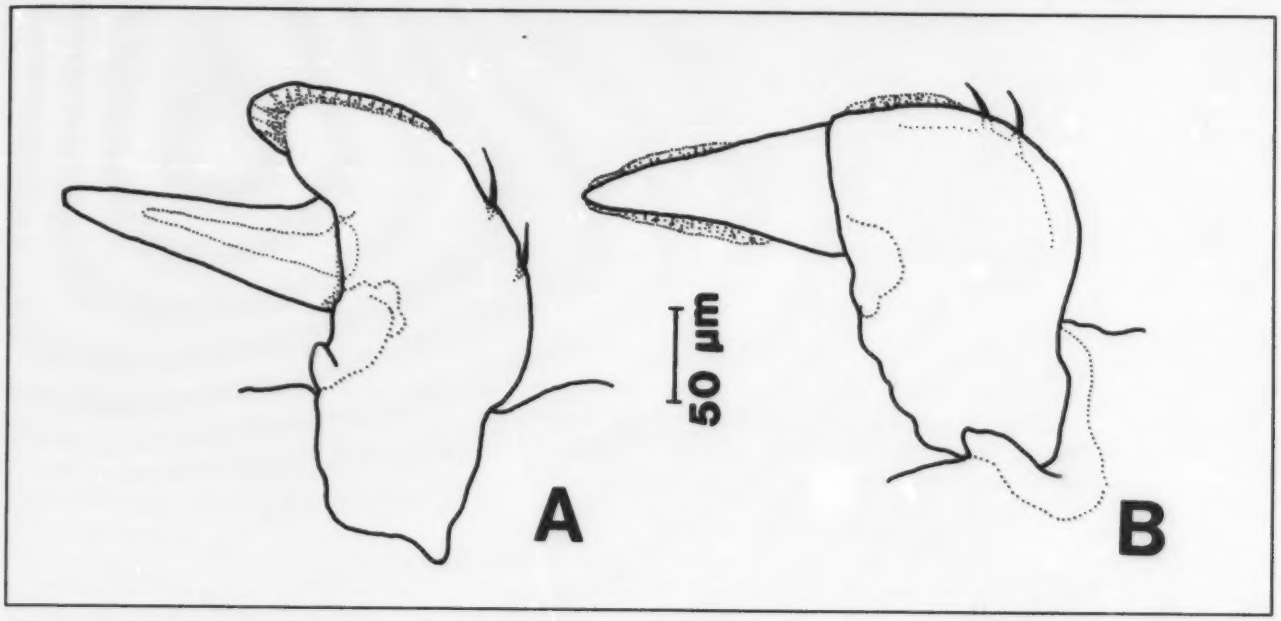


Figure 32. Basal spine of the exopod of the adult third leg: A, L. cuneifer*; B,* L. salmonis*. Note that in both species the exopod of the third leg has attained the adult form by the first preadult stage. (From Johnson and Albright 1991a).*

swimming infectious copepodid stage, four attached chalimus stages, two free-moving preadult stages, and one free-moving adult stage (Fig. 33) (Kabata 1972; Johnson and Albright 1991b). Attached copepodids, chalimus larvae, preadults, and adults feed on host mucus, skin, and blood (Kabata 1970, 1974a; Brandal et al. 1976; Jones et al. 1990).

Sea lice have been reported to cause serious disease in many seawater netpen facilities and in some seawater land-based facilities (Brandal and Egidius 1979; Wootten et al. 1982; Hogans and Trudeau 1989a,b; Pike 1989; Tully 1989; unpublished data for British Columbia). Important biological and environmental factors that may determine the severity of infections include: water temperature, presence of reservoir hosts, water circulation, salinity, species cultured, general fish health, and stocking density. However, few of these factors have been sufficiently investigated.

In general, prevalence of sea lice increases with higher temperatures, independent of time of season (Wootten et al. 1982; Hogans and Trudeau 1989b; Tully 1989). Generation times of sea lice are temperature dependent. The generation time of *L. salmonis* is approximately 6 wk at 9 - 12 °C (Wootten et al. 1982) and 7.5 wk at 10°C (Johnson and Albright 1991c).

Egg-bearing females of *C. clemensi* and *L. salmonis* are present throughout the year. Hatching of the eggs and development to the infective copepodid stage in both species occurs at temperatures as low as 5 °C (Johnson and Albright 1991c; unpublished data).

The presence of wild hosts may serve as a reservoir of sea lice. Many non-salmonid hosts of *C. clemensi* and *L. cuneifer* are often attracted to and present in large numbers in the vicinity of netpen sites. Wild hosts were the source of *C. clemensi* that infected salmonids in a seawater tank facility in British Columbia. Some evidence suggests that the severity of infections at particular sites increases with time but this is yet to be documented (see Wootten et al. 1982). Additional information on the biology of sea lice is given in Wootten et al. (1982) and Pike (1989).

CLINICAL SIGNS AND GROSS PATHOLOGY. Fish with heavy sea lice infections commonly show grey patches (extensive areas of skin erosion) and hemorrhaging on the head and dorsal area, and a distinct area of erosion, dark coloration, and sub-epidermal hemorrhages in the perianal region (Wootten et al. 1982). Focal, shallow ulcers are also seen on the flanks (Fig. 34, pg. 55). Massive ulcers with exposed muscle are commonly reported in heavy infections of *L. salmonis* in European fish farms. In the Pacific Northwest such ulcers have not been reported in pen-reared salmonids, but similar lesions to those reported on pen-reared salmonids in Europe have been seen on wild sockeye salmon returning to spawn in British Columbia waters. Death may be

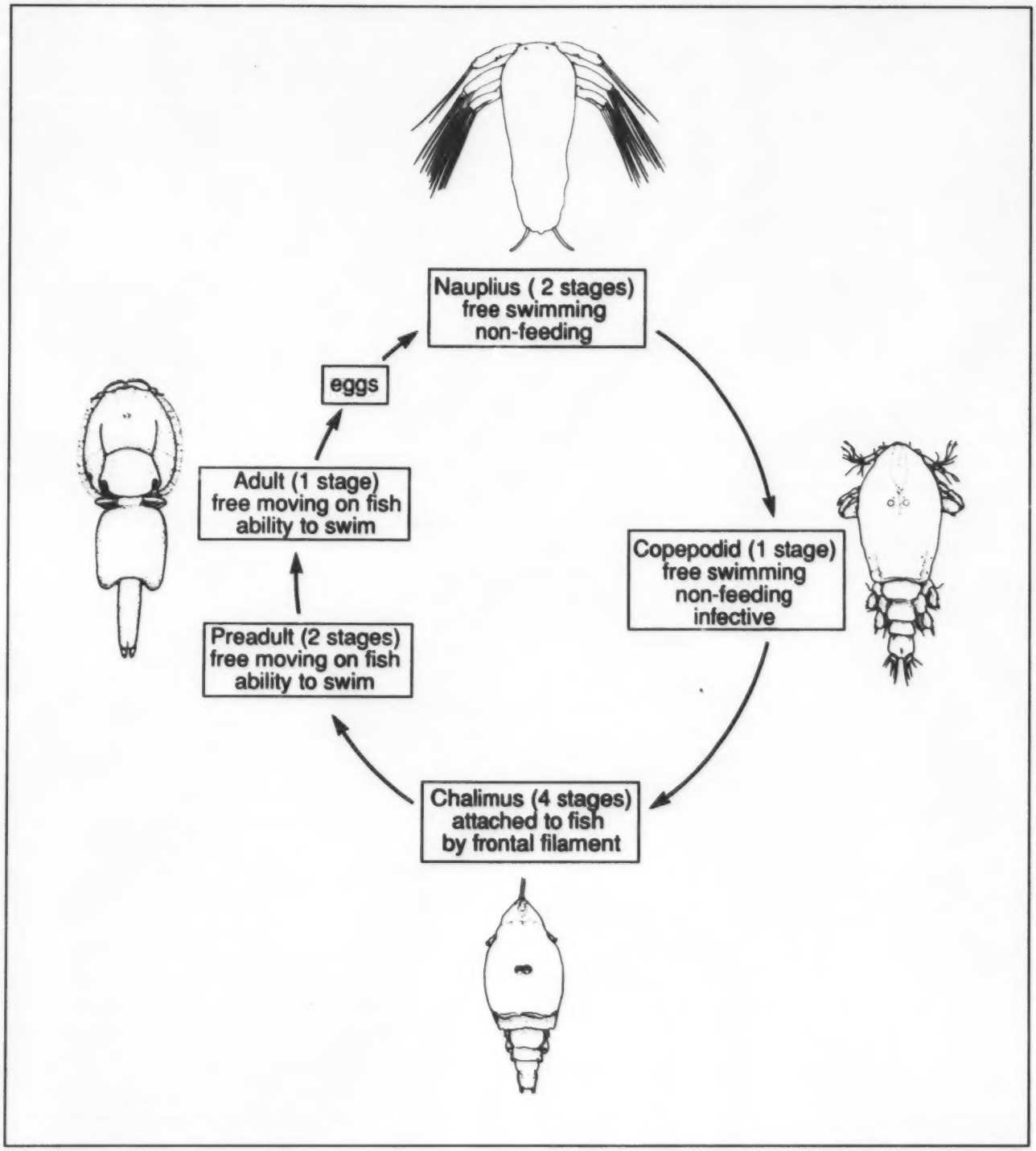


Figure 33. The life cycle of sea lice.

caused by secondary bacterial infections (e.g., vibriosis), by fungal infections when fish are re-introduced to fresh water, or by osmotic stress (Wootten et al. 1982).

Lepeophtheirus salmonis is a relatively large parasite (up to 10 mm excluding egg strands) and is easily seen on the surface of the fish, particularly in heavy infections. Both *C. clemensi* and *L. cuneifer* are smaller (up to about 5 mm) and require closer examination to see the parasite

with the naked eye. The lesions associated with heavy *C. clemensi* infections are similar to those of *L. salmonis*, except that we have not observed focal ulcers that extend through the dermis in the former. Heavy infections of *C. clemensi* on the fins of pink salmon resulted in various stages of fin damage up to complete removal (Parker and Margolis 1964). Studies on leaping behaviour in salmon suggest a relationship between the presence of sea lice and leaping frequency, but the evidence is not conclusive (Furevik et al. 1988, cited in Pike 1989).

MICROSCOPY. Wet mount preparations of skin scrapings examined under a dissecting microscope will demonstrate the developmental stages of the parasite (Fig. 33). Histological examination of tissue sites of copepod feeding reveals that the epidermis of Atlantic salmon is eroded, and the dermis exhibits haemorrhagic areas (Wootten et al. 1982). Fins of Atlantic and chinook infected with chalimus larvae of *L. salmonis* are eroded. In contrast, the skin and fins of coho infected with the chalimus larvae of *L. salmonis* show a pronounced epithelial hyperplasia and an inflammatory infiltrate. (Johnson 1991).

DIAGNOSIS. Diagnosis is confirmed by observing the copepods, and determining their specific identity through microscopic examination. The small size of the copepodid (about 1 mm) and chalimus (about 1 to 3 mm) stages requires the use of a dissecting microscope to detect their presence. Easily recognized features used for distinguishing the preadult and adult stages of the three species of sea lice found on pen-reared salmonids in the Pacific Northwest include the presence or absence of lunules, body shape, and structure of the third leg. General body shape can be determined using a dissecting microscope. Determining the structure of the third leg requires its removal and examination under a compound microscope.

In general, the preadult and adult stages of *C. clemensi* can be easily distinguished from those of *L. salmonis* and *L. cuneifer* by the presence of lunules (Fig. 31). Identification of preadult and adult *C. clemensi* can be confirmed by reference to Kabata (1972, 1988) and Parker and Margolis (1964). Preadults and adults of *L. salmonis* can be distinguished from *L. cuneifer* by differences in the structure of the basal spine of the exopod of the third leg (Fig. 32). Furthermore, *L. salmonis* adults can be easily recognized from *L. cuneifer* adults by the shape and relative size of the genital complex and abdomen. Identification of preadult and adult stages of *L. salmonis* can be confirmed by reference to Kabata (1973, 1979, 1988) and Johnson and Albright (1991b). Identification of adult *L. cuneifer* can be confirmed by reference to Kabata (1974b).

It is possible to identify the copepodid and chalimus stages of *C. clemensi* and *L. salmonis*, but their identification is difficult and requires a detailed knowledge of copepod structure. These developmental stages of *C. clemensi* and *L. salmonis* can be identified by reference to Kabata (1972) and Johnson and Albright (1991b), respectively. The earlier developmental stages of *L. cuneifer* have not been described.

The following key serves to distinguish the preadults and adults of the three species of sea lice reported from sea-farmed salmon and trout in the Pacific Northwest.

1) Lunules present ..... *C. clemensi* (adults Figs. 31E, 31F)
   Lunules absent 2

2) Basal spine of third exopod at some distance from tip of basal swelling (Fig. 32A) .... *L. cuneifer* (adults Figs. 31A, 31B)

   Basal spine of third exopod at tip of basal swelling (Fig. 32B) .... *L. salmonis* (adults Figs. 31C, 31D).

CONTROL AND TREATMENT. At present the only effective method for control of sea lice on farmed salmonids is the organophosphate insecticide, dichlorvos, marketed as 'Nuvan 500EC' or in its related trichlorphon form as 'Neguvon'. Brandal and Egidius (1977) reported the first use of trichlorphon for the treatment of salmonids infected with sea lice. In their study, trichlorphon, which was administered orally resulted in a decline in the number of sea lice present and a high level of mortality in the treated fish. Dichlorvos and trichlorphon have been used since the 1960's as a bath treatment for parasites in pond fish culture (reviewed in Schmahl et al. 1989). Currently these compounds are administered to salmonids as a bath treatment following the methods described by Brandal and Egidius (1979). These treatments effectively remove both the preadult and adult stages of sea lice but not the chalimus larvae; therefore, successive treatments usually at two to four week intervals are required to control infections (Wootten et al. 1982). Physical damage and high levels of stress imposed during treatment may cause secondary diseases (e.g.,

vibriosis, furunculosis) in the fish. In addition, production levels of treated stocks are lower due to lowered growth and feed conversion rates.

About 12,000 litres of 'Nuvan 500EC' per year are used to control sea lice in Scotland (Pike 1989). Estimates for other countries are not available. Although, these compounds are recognized as toxic to a wide variety of marine organisms, they are released into the surrounding waters after treatment is completed, where it is argued that their impact on non-target species is minimal due to the rate of dilution and their rapid breakdown (Egidius and Moster 1987; Cusack and Johnson 1990; Dobson and Tack 1991). This practice has resulted in conflict between environmental groups and salmon farmers over the use of these chemicals and has led Norway, Scotland, and Ireland to consider banning their use. These compounds are legally unavailable for use in Canada according to present legislation.

There is little published information on alternative treatment methods for sea lice. Palmer et al. (1987) reported the results of preliminary studies on the efficacy of oral doses of Ivermectin for the control of sea lice on Atlantic salmon. Although this drug was found to be effective in reducing populations of sea lice, the drug had a narrow margin of safety. Studies conducted in our laboratory showed that Ivermectin can be very toxic to Atlantic salmon. Fish fed 0.05 mg/kg on alternate days became anorexic after 20 days, and Ivermectin was lethal to fish fed higher doses. Fish suffering from Ivermectin toxicity are listless, show ataxia, and then die in a few days. Further research on the efficacy of Ivermectin and on residues of the drug in tissue needs to be done before this drug can be considered for use as a sea lice treatment.

Bjordal (1988, 1990) reported the results of both laboratory and field studies involving the use of cleaner wrasses (family Labridae) for controlling *L. salmonis* on Atlantic salmon. In these studies wrasses removed sea lice from salmonids but not always in a predictable manner. A survey of fish farmers in Scotland that have tried wrasses to control sea lice showed that the majority felt that this sea lice control measure was benificial, particularly when used in conjunction with Dichlorvos treatments (Anonymous 1991). However, problems were encountered using wrasses to control sea lice. A smaller mesh size in nets was required to prevent their escape, and some wrasses were intimidated by larger salmon and tended not to clean them, while other wrasses became aggressive and inflicted scale and eye damage to the salmon. In addition, some farmers feared that the wrasses ate salmon feed and would abandon their cleaning behavior (Anonymous 1991). The wrasse species used in these studies do not occur naturally in the Pacific Northwest and therefore they must be imported and/or cultured.

Scottish researchers are investigating the possibility of producing a vaccine to control sea lice, Norwegian researchers are investigating the use of pyrethrins administered as a bath treatment (Boxaspen and Holm 1991), and some fish farmers have suggested that hanging sacks of onions in pens can control the infection. The efficacy of these unconventional treatments have not been established.

Figure 34A

Figure 34B

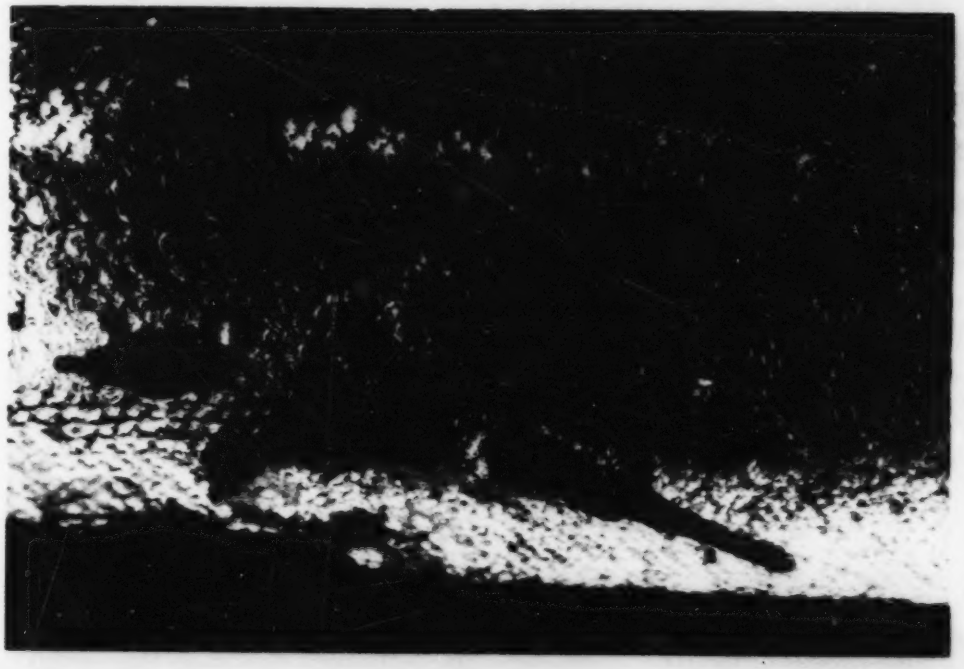


Figure 34C

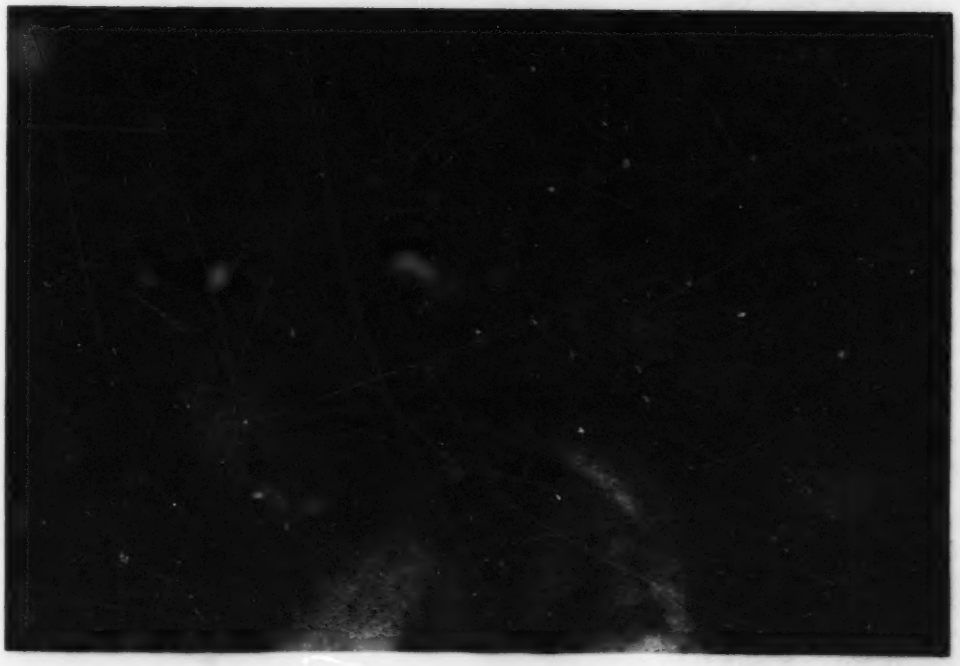


Figure 34D

Figure 34. A. Anisakis *third stage larvae from the viscera of a Pacific herring. B.* Neascus *(black spot) metacercariae in the skin of a coho. C. Sea lice,* Lepeophtheirus salmonis, *and associated focal ulcer in the skin of an Atlantic salmon. D. Gill of Atlantic salmon from* Chaetoceros *diatom bloom. Hyperplastic epithelial tissue appears as a large, whitish mass in the gills. (Courtsey of R.A. Elston).*

ALGAL BLOOMS

Algal blooms have caused fish kills in coastal waters throughout the world in both wild and captive marine fishes (Möller and Anders 1986; Gaines and Taylor 1986; White 1988). In salmon and yellowtail cultured in sea water, serious losses have been caused by toxin producing algae such as *Heterosigma akashiwo* (Okaichi 1983; Gaines and Taylor 1986; Rensel et al. 1989; Black et al. 1991), *Gonyaulax excavata* (Mortensen 1984), *Gyrodinium aureolum* (Tangen 1977; Jones et al. 1982), *Chrysochromulina polylepis* (Saunders 1988), and *Chattonella antiqua* (Gaines and Taylor 1986). In the Pacific Northwest, blooms of the flagellate *H. akashiwo*, and three diatoms, *Chaetoceros convolutus* and *C. concavicornus*, and a *Corethron* species, have been associated with mortality in pen-reared fishes (Gaines and Taylor 1986; Speare et al. 1989; Rensel et al. 1989; Horner et al. 1990; Black et al. 1991). Approximately $ 11 million of pen-reared salmon were lost to algal blooms in B.C. in 1986 through 1989. There are four known general mechanisms in which algal blooms may kill fish (Black et al. 1991): physical damage to the gills by the spines of diatoms such as *Chaetoceros* spp., asphyxiation caused by oxygen depletion, gas-bubble trauma due to extreme oxygen saturation from algal photosynthesis, and direct chemical toxicity caused by icthyotoxins

Heterosigma akashiwo

Several blooms of *Heterosigma akashiwo* (=*Olisthodiscus luteus*) have been associated with high mortality in pen-reared salmon in the Pacific Northwest since 1986. Fish kills associated with *H. akashiwo* blooms have also been reported in Europe, Japan, and New Zealand (Gaines and Taylor 1986; White 1988; Chang et al. 1990).

Experiments conducted by Black et al. (1991) indicate that the most likely cause of morbidity in fish is due to an unidentified, labile toxin. Blooms generally occur during the summer, and massive mortalities have been observed in all species of pen-reared salmon throughout B.C. (Egan 1989a; Stockner 1989) and Washington (Egan 1989b; Rensel 1990). High mortalities were observed in pen-reared chinook during two separate blooms when the concentrations of *H. akashiwo* in the water were 200,000 and 800,000 cells/ml (Black et al. 1991). A concentration of approximately 20,000 cells/ml was associated with mortality in Washington (Rensel 1990)

CLINICAL SIGNS AND GROSS PATHOLOGY. Rapid, high mortality (often reaching near 100 %) occurs shortly after water containing a bloom enters the netpens. Affected fish accumulate at the surface in the net corners, are lethargic and exhibit labored respiration. Fish do not exhibit any distinctive external or internal pathological changes. However, some affected fish may exhibit excessive mucus on the gills.

MICROSCOPY. In wet mount preparations, the alga appears as small, motile, bean-shaped organisms (Fig. 35). The following is a description of *H. akashiwo* by Gaines and Taylor (1986):

> "The organism occurs as microscopic, bean shaped single cells, usually 12-22 µm long, propelled by two very fine, whip-like flagella which arise from the side of the cell near the front, one pointing forwards and one trailing behind. The cells turn slowly as they swim forward. The shape varies from being smoothly oval (flattened in the same plane as the flagella) to being bumpy and potato-like in outline. Each cell contains usually 20 or fewer (but may have up to 50) greenish-brown bodies termed chloroplasts.... ... Small shining dots on its surface are mucus-producing bodies, termed mucocysts".

The organism is greatly distorted by preservation in Lugol's solution (see Appendix III), a preservative that is routinely used to preserve phytoplankton (Gaines and Taylor 1986). In Lugol's preservative the cell membrane

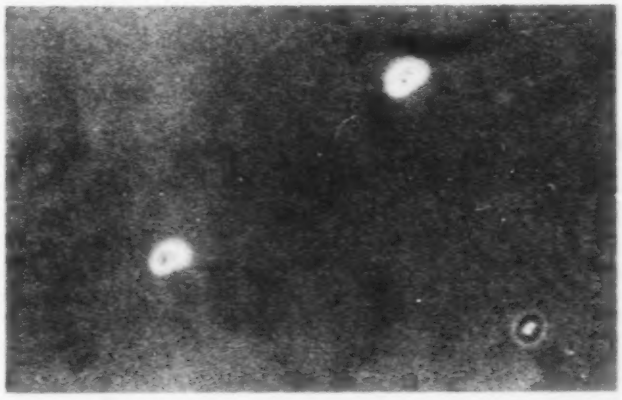


Figure 35. Wet mount of Heterosigma akashiwo. *Phase contrast.*

collapses and the cell appears as a gold-stained crinkled corn flake or a cluster of grapes.

The histopathological changes in fish dying from exposure to *H. akashiwo* have not been adequately described. We have observed acute, diffuse necrosis of the gill epithelium in dying fish collected from the field during a *Heterosigma* bloom. These fish were suffering the affects of the bloom for several hours before collection, and this change was not observed in fish that were killed by more acute exposure (Black et al. 1991).

DIAGNOSIS. Because moribund fish do not exhibit distinctive pathological changes, the diagnosis of disease caused by *H. akashiwo* is based on the observation of a high concentration of the alga in the water associated with rapid mortality in the exposed fish. Methods for collecting and enumerating planktonic algae are described by Gaines and Taylor (1986).

CONTROL AND TREATMENT. There are no known therapeutic agents for treating fish exposed to *Heterosigma.* General strategies used to ameliorate the affects of noxious plankton blooms have had some success with reducing losses associated with blooms. These include not feeding fish during blooms and minimizing disturbances on the pens (eg., keeping personnel off pens). Avoiding high concentrations of the algae has been achieved by moving pens away from an encroaching bloom, pumping water from deeper depths, encircling the pens with a plastic curtain to impede water movement, and providing fish with deep pens so that fish can swim below the bloom.

Chaetoceros and *Corethron* spp.

Chaetoceros convolutus, C. concavicornus and a *Corethron* sp. have been associated with mortality in pen-reared salmon in the Pacific Northwest. Mortalities due to *Chaetoceros* spp. have been reported in pen-reared salmon at many sites in B.C. and Washington (Bell et al. 1974; Gaines and Taylor 1986; Horner et al. 1989; Rensel et al. 1989), and Alaska (Martin 1981). Mortality caused by *Chaetoceros* is usually very rapid, and high mortality can occur within a few days during heavy blooms. All salmon species are susceptible. Atlantic salmon and sockeye salmon appear to be the most susceptible species, and larger fish are more susceptible than smaller fish (Brett et al. 1978; Marsh 1988). Speare et al. (1989) reported high mortality in pen-reared coho associated with a bloom of *Corethron* sp., a diatom similar to *Chaetoceros.* Both genera of diatoms cause disease in fish through physical damage to the gill (Bell 1961; Farrington 1988; Speare et al. 1989).

In netpens, mortality in sockeye salmon associated with *Chaetoceros* blooms occurred at 1 - 5 cells/ml (Kennedy et al. 1976). High mortality occurred in sockeye, coho and chinook during a bloom of *Chaetoceros* at 8-32 cells/ml (Brett et al. 1978), and 8 cells/ml killed Atlantic salmon in netpens (Marsh 1988). In laboratory experiments 100-150 cells/ml were required to achieve an LC50 in chinook and chum salmon (Farrington 1988). The high concentration of *Chaetoceros* that is required to kill salmon in the laboratory has been attributed to a loss of spines on the setae when the alga is grown in culture.

CLINICAL SIGNS AND GROSS PATHOLOGY. Typical of gill diseases, affected fish accumulate near the surface, are lethargic, and exhibit labored respiration and flaring of the opercula. Examination of gills reveals excessive mucus and may reveal white patches of hyperplastic tissue at the base of the primary filaments, particularly at the bend in the gill arch (Fig. 34, pg. 55). The internal organs of affected fish appear normal.

MICROSCOPY. Wet mount preparations of the gills may reveal masses of diatoms entrapped in the mucus (Fig. 36). Histological examination of the gills reveals severe hyperplasia and necrosis of the epithelium (Fig. 36). Hyperplasia of the epithelium results in fusion of the secondary lamellae, and in severe cases, the primary lamellae may be fused. Affected gills often exhibit prominent inflammation. Speare et al. (1989) observed a marked neutrophil response in the extravascular spaces and in the lymphatic-like central venous sinusoidal spaces of the gills. Diatoms can be found on the surface of the gill epithelium or embedded in the hyperplastic tissue, particularly at the base of the primary lamellae (Fig. 36). The embedded portions of the diatoms may be entrapped by multinucleate giant cells. Mild fibroplasia may be observed associated with diatom remnants in recovering fish (Speare et al. 1989).

DIAGNOSIS. Diagnosis of *Chaetoceros* or *Corethron* disease can be based on the occurrence of large numbers of the segmented diatoms with spiny setae in the water associated with respiratory distress in the salmon. Histological examination, which reveals the presence of

entrapped diatoms and associated gill damage, is also useful.

CONTROL AND TREATMENT. As with *Heterosigma* blooms, suspending feeding fish and other fish farming activities that may excite the fish or bring them to the surface during blooms may reduce mortalities. Avoiding high concentrations of the algae has been achieved with limited success by pumping water from deeper depths, encircling the pens with a plastic curtain to impede water movement, and providing fish with deep pens so that fish can swim below the blooms.

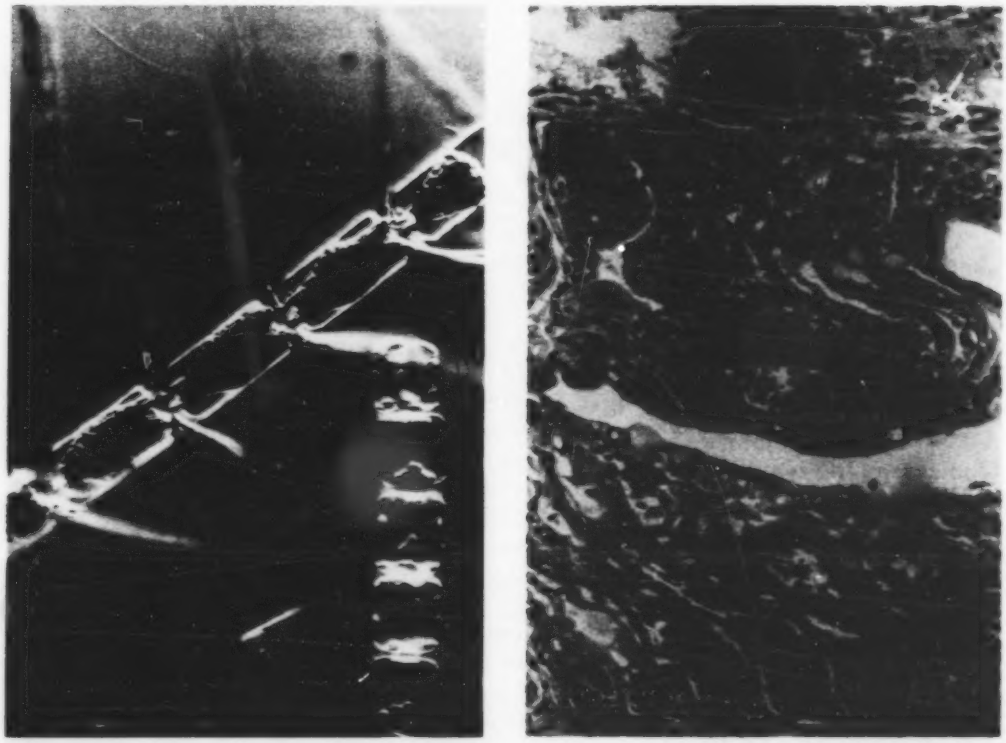


Figure 36. Chaetoceros *diatoms from salmon gills. A. Wet mount of* Chaetoceros *sp. from the gills of Atlantic salmon. (Courtesy of R. A. Elston). B. Hyperplasia of the gill epithelium due to entrapped* Chaetoceros *algae (arrow). H & E.*

IDIOPATHIC DISEASES

Several diseases of unknown causes have afflicted pen-reared salmon in the Pacific Northwest. These include plasmacytoid leukemia (also known as marine anemia) caused by an undetermine infectious agent; pancreas disease, possibly caused by a virus; netpen liver disease, possibly caused by a toxin; and "water belly", which may be caused by a nutritional or osmoregulatory problem.

Plasmacytoid leukemia

A plasmacytoid leukemia (PL) of chinook, referred to as marine anemia by fish farmers, has caused high mortality at many netpen farms in British Columbia (Kent et al. 1990a; Newbound and Kent 1991b). Plasmacytoid leukemia is characterized by infiltration and proliferation of immature plasma cells (plasmablasts) in the visceral organs and retrobulbar tissue. The disease is usually detected in chinook after they have been in sea water for approximately one year, and outbreaks are often recognized following BKD epizootics. Mortalities associated with PL have varied considerably, and at least one farm lost approximately 50% of the production stock in the fish's second year in sea water.

The etiology of PL has not been determined, but field observations and laboratory transmission studies clearly indicate that the disease is caused by an infectious agent (Kent and Dawe 1990; Newbound and Kent 1991a). Plasmacytoid leukemia of chinook is readily transmitted by injection of homogenates of affected kidney and spleen tissue. In addition, we have experimentally infected sockeye salmon and Atlantic salmon by injection of affected chinook tissues (Newbound and Kent 1991a). Two agents have been suggested to be the cause of PL; a microsporidian parasite, *Enterocytozoon salmonis* (pg. 42), and an oncogenic virus (e.g., retrovirus). The microsporidium has been observed in the nuclei of the plasmablasts from many fish with PL in B.C. However, the experimental evidence suggests that the microsporidium is not the primary cause of PL, and that an oncogenic retrovirus is the cause of the disease (Eaton et al. 1991; Kent et al. 1991a).

Essentially all infectious leukemias and related disorders are caused by oncogenic viruses (particularly retroviruses) and these have been associated with some blood neoplasms of fishes (Papas et al. 1976; Gross 1983). Virus-like particles similar to retroviruses have been detected in affected fish (Kent et al. 1991a), and Eaton et al. (1991) consistently found increased reverse transcriptase activity, a retroviral enzyme, in fish with PL.

Non-viral agents, including parasites, can be co-factors or promoters for virus-induced neoplasms (Gross 1983). Possibly, *E. salmonis* is a co-factor in PL that promotes the replication of the oncogenic virus. Interestingly, the only other member of the genus *Enterocytozoon*, *E. bieneusi*, is associated with a retroviral disease, AIDS in humans (Desportes et al. 1985; Bryan et al. 1990). *Renibacterium salmoninarum*, the cause of BKD, may also be a co-factor in PL. However, as with *E. salmonis*, laboratory transmission studies indicate that the bacterium is not the primary cause of the disease. Given the high prevalence of BKD that proceeds most outbreaks of PL, it is possible that the bacterium may exacerbate the proliferation of plasmablasts seen in PL. Conversely, PL-affected fish may be predisposed to BKD.

There are many unanswered questions about PL and similar diseases of freshwater-reared chinook in California (Hedrick et al. 1990) and Washington (Harshbarger 1984; Morrison et al. 1990). It is not known if the diseases in the USA are the same as PL in B.C., the primary cause of PL is yet to be confirmed, and the role of the microsporidium in PL is unclear. An understanding of the epizootiology of PL is also lacking. It is not known if PL occurs in freshwater-reared chinook in B.C., nor is it known how the disease is transmitted. Transmission studies suggest that PL is not transmissible in sea water, or is at least not highly transmissible in this environment. Recent observations suggest that the disease can be transmitted vertically through infected eggs.

CLINICAL SIGNS AND GROSS PATHOLOGY. Affected fish are dark, lethargic, and often swim near the surface. Pallor of the gills, indicating anemia, is a common finding. Many of the fish with PL exhibit severe bilateral exophthalmus (Fig. 40, pg. 66). Some of these fish may exhibit severe eye involvement with relatively minimal visceral changes and anemia. Dissection of affected eyes reveals that the exophthalmus is due to massive accumulation of white or hyperemic tissue in the orbit of the eye. Internal examination reveals enlargement of the spleen and kidney (Fig. 40). Petechiae may occur in the liver, mesenteric fat, pancreas, heart and skeletal

Figure 37. Plasmacytoid leukemia in chinook salmon. H & E. (Courtesy of Dis. Aquat. Org.).
A. Hyperplasia of the kidney interstitium caused by proliferation of plasmablasts.
B. Proliferation of plasmablasts in the ocular muscle.
C. Plasmablast proliferation in pancreas and associated fat. A = pancreatic acinar cells.

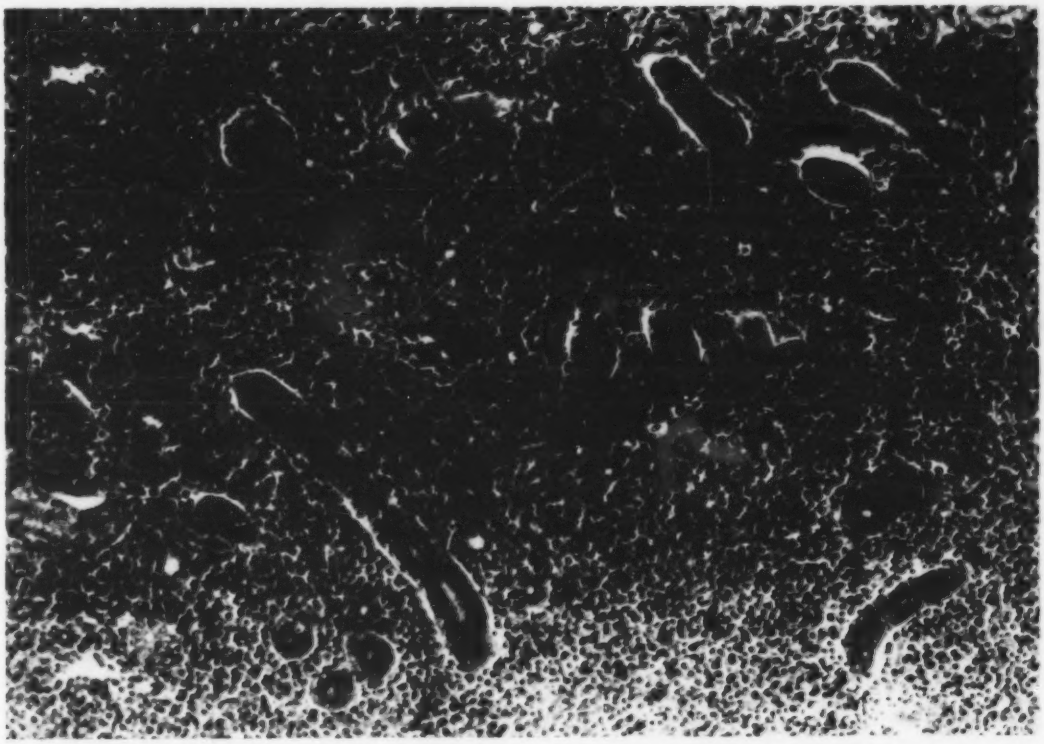


Figure 37A

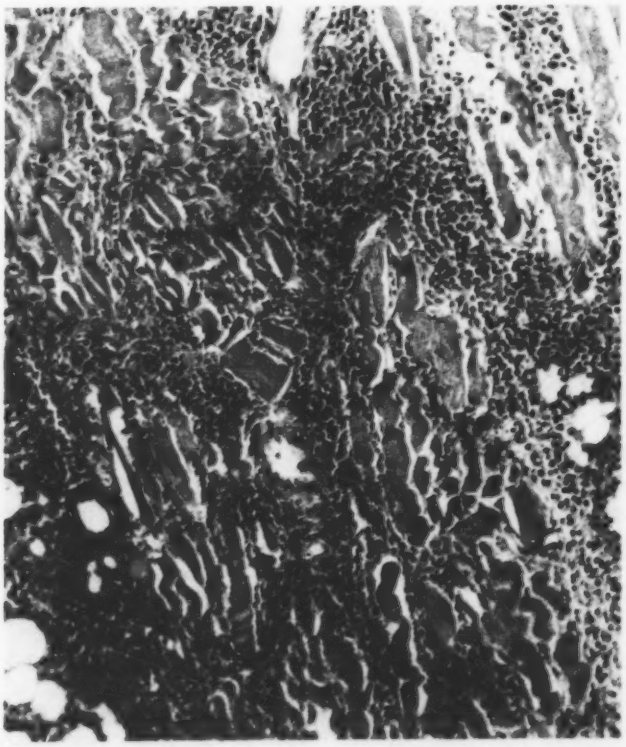


Figure 37B

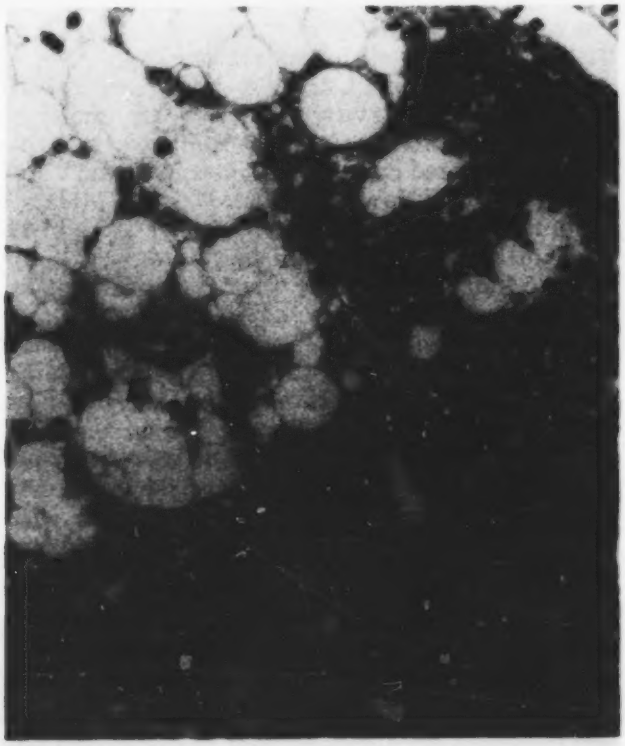


Figure 37C

muscle. The lower intestinal wall may be markedly thickened. Some fish have ascites consisting of a clear or serosaguinous fluid. Hematocrit values of blood from affected fish are quite variable, and may reach as low as 2%.

MICROSCOPY. Histological examination of affected fish reveals a proliferation of plasmablasts essentially in every organ (Figs. 37), including kidney, spleen, liver, intestine, pancreas and associated mesenteric fat, meninges, heart, skeletal muscle, skin, and the eye (Kent et al. 1990a). In paraffin sections, the plasmablasts contain large, often deeply clefted or lobated nuclei, and prominent nucleoli. They have a moderate amount of finely granular, eosinophilic or amphophilic cytoplasm, and many of these cells are mitotically active. The eye, spleen, and kidney are the primary organs affected.

In the eye, there is massive infiltration of plasmablasts into the periorbital connective tissue and ocular muscles (Fig. 37). Kidneys exhibit prominent hyperplasia of the interstitium due to proliferation of the plasmablasts (Fig. 37). Thickened basement membranes in the capillaries of the glomerulus are often observed. In severely affected fish, there is a perivascular infiltration of the plasmablasts within the liver, and the cells often proliferate within the sinusoids.

The pericardium of the ventricle, atrium, and bulbus arteriosus of the heart may be infiltrated by the plasmablasts, which forms a thick cellular capsule that surrounds the heart. The cells may also infiltrate the endocardium, particularly in the bulbus arteriosus, and plasmablasts occur throughout the vascular sinuses of the heart. When the lower intestine is affected, there is massive proliferation of the plasmablasts in the lamina propria and in the submucosa, resulting in expansion of the intestinal villi.

Giemsa-stained imprints of kidney, liver, and the eye reveal numerous plasmablasts (Fig. 38). In these preparations, the cells have a smooth contour, vary in size from 10 to 20 µm, and contain sparse to moderate amounts of basophilic cytoplasm. Nuclei are large, occasionally indented, and contain a smoothly homogeneous to moderately reticulated chromatin pattern. A juxtanuclear hof is visible in some cells.

DIAGNOSIS. Diagnosis of PL based on gross pathological changes is impossible because these changes are very similar to those of fish with BKD. Presumptive diagnosis and distinction from BKD can be achieved by the examination of Giemsa-stained and Gram-stained imprints of the liver and kidney. Examination of an organ that is normally not a site of hemopoiesis, such as the liver, is recommended because differentiation of abnormal populations of plasmablasts from

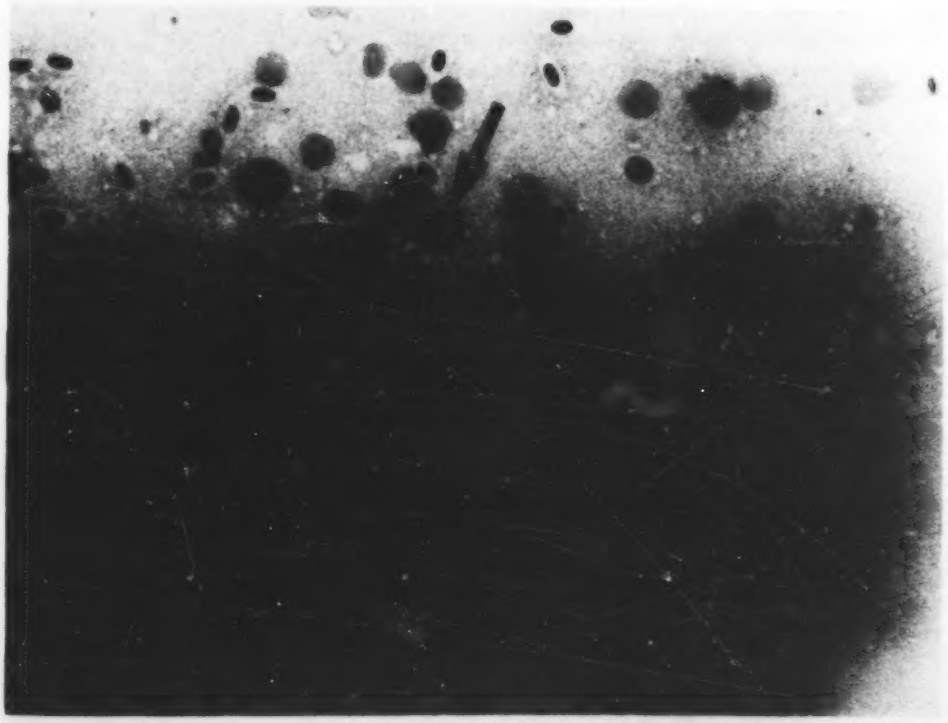


Figure 38. Liver imprint stained with Giemsa. Arrow = plasmablasts, L = hepatocytes.

normal hemoblasts in organs such as the spleen and kidney may be difficult. The occurrence of large numbers of immature blood cells (presumably plasmablasts) in the liver (Fig. 38), and the absence of numerous *Renibacterium* organisms in the kidney and liver allows for a presumptive diagnosis of PL. However, a false negative diagnosis can occur using these criteria alone because PL-affected fish may exhibit concurrent BKD, and fish with PL do not always exhibit numerous plasmablasts in the liver.

Therefore, confirmatory diagnosis should be based on histological examination. Diagnosis by histology is based on the occurrence of renal interstitial hyperplasia of cells resembling the plasmablasts, and proliferation of these cells in at least one non-hemopoietic organ. Concurrent BKD can also confuse the histological diagnosis. The severe, chronic inflammatory response that occurs throughout the viscera and occasionally the eye in fish with severe BKD can mask the detection of the plasmablast infiltrates.

CONTROL AND TREATMENT. The mode of transmission of PL and the source of the infection in the field is still unclear. Therefore, precise recommendations for the control of the disease at affected sites are not available. Given the infectious nature of PL and its ability to cause high mortality, fish should not be transferred from sites with PL to sites where the disease has not been found. Until the risk of vertical transmission has been further evaluated it would be prudent not to use eggs or smolts that originated from brood stock with a history of PL. If this is not possible, the individual spawners should be examined, and eggs and sperm from PL-positive fish should be discarded.

There are no commercially available anti-leukemia drugs for fish. However, Hedrick et al. (1991) reported that treatment of a disease similar to PL in California with Fumagillin, an anti- microsporidial drug, reduced infections by *E. salmonis* and the concurrent leukemia-like condition. This indicates that, although *E. salmonis* is not the primary cause of PL, elimination of potential co-factors, such as *E. salmonis* infections and BKD, may be helpful for the control of PL. However, the usefulness of this drug for treating PL is questionable because control of the disease with Fumagillin in experimentally infected fish in our laboratory was unsuccessful.

Netpen Liver Disease

A toxicopathic liver disease has been observed in Atlantic salmon at several netpen sites in Washington and B.C. (Kent et al. 1988b; Kent 1990). The disease, referred to as 'netpen liver disease' or NLD, affects Atlantic salmon during their first year in sea water. The disease begins in the summer, usually is chronic with steady, persistent mortality, and may persist through the early autumn. Fish that survive until the winter recover. Mortality associated with the disease is extremely variable; fish from some sites may exhibit only mild to moderate liver damage and no significant increase in mortality, whereas in other epizootics there has been severe liver damage and almost 90% cumulative mortality by the end of the summer.

Although the etiology of NLD is unknown, the cause is most likely a naturally occurring toxin from the netpen environment. The liver lesions (e.g., hepatic megalocytosis) are similar to those caused by other hepatotoxicants. Observations and in vivo laboratory experiments have indicated that NLD is not caused by an infectious agent, that it is not associated with anthropogenic (man-made) contamination, and that it is not directly related to contamination of commercial feed (Kent et al. 1988b; Kent 1990). Our present hypothesis is that the agent may be a hepatotoxin that is produced by a naturally occurring organism, perhaps an alga, that is abundant in netpens during the summer. Atlantic salmon that feed heavily on netpen biota, particularly *Caprella* spp. (Amphipoda) and copepods, often exhibit NLD. Therefore, it is possible that fish are exposed to the NLD agent by feeding on invertebrates that have accumulated the toxin. Atlantic salmon are the most susceptible species, but NLD has also been observed in chinook and rainbow trout - steelhead crosses (Kent 1990). We have also observed essential identical liver lesions in about 5 % of the wild-caught chinook from the Strait of Georgia, B.C.

CLINICAL SIGNS AND GROSS PATHOLOGY. Affected fish may be dark, lethargic and accumulate in the netpen corners. The liver of affected fish may be small, yellow, opaque, and friable (Fig. 40, pg. 67). However, fish can have severe liver damage without any obvious macroscopic changes to the liver. Other organs appear normal.

MICROSCOPY. The hallmark histological change of NLD is prominent hepatic

megalocytosis (Fig. 39). Affected livers also exhibit a variety of other pathological changes. Early in the disease the liver shows vacuolation of hepatocytes, individual cell necrosis of hepatocytes scattered throughout the parenchyma (hepatocyte dropout), nuclear pleomorphism of hepatocytes, and bile preductual cell proliferation. Moderate and severe lesions are characterized by prominent nuclear pleomorphism leading to megalocytosis, diffuse necrosis and hydropic degeneration of hepatocytes, perivascular and peritubular cuffing by inflammatory cells, and ceroid deposition in macrophages.

Fish that survive NLD exhibit islands of hepatocyte regeneration. After affected fish are moved to clean water they exhibit almost complete regeneration of the liver in 3 mo. (Kent 1990). However, individual hepatocytes with megalocytosis can be found in regenerated livers at least 9 mo. after fish are moved to clean water. Therefore, the presence of these megalocytic hepatocytes in fish collected during the winter can be used as an indication that the fish previously had NLD.

DIAGNOSIS. Diagnosis of NLD is based on the presence of the histological changes described above in pen-reared salmon. The hallmark of NLD is hepatic megalocytosis. However, this change can be induced by a variety of hepatotoxins and may be found in fish with other liver diseases.

CONTROL AND TREATMENT. There is no known treatment for the disease aside from transferring fish out of affected netpens.

Figure 39A

Figure 39B

Figure 39. Atlantic salmon livers with netpen liver disease. H & E.
A. Hydropic degeneration of hepatocytes and island of regenerating hepatocytes (arrows). B. Hepatic megalocytosis. Note extremely hypertrophied nuclei (arrows).
(Courtesy of Dis. Aquat. Org.).

Pancreas Disease

Pancreas disease of pen-reared Atlantic salmon is a prevalent and severe disease in Europe (Munro et al. 1984; Ferguson et al. 1986a; McVicar 1987). The disease has also been observed in pen-reared Atlantic salmon in Washington State (Kent and Elston 1987) and one outbreak has occurred in B.C. The cause of the disease has been controversial. Laboratory transmission studies indicate that the disease is caused by an infectious agent (McVicar 1990), whereas as the clinical and histopathological changes observed in affected fish were suggestive of a vitamin E - selenium deficiency (Ferguson et al. 1986 a,b). Bell et al. (1987) concluded that the depletion of vitamin E and selenium observed in affected fish is probably an effect of the disease rather than its cause.

Pancreas disease occurs only in salmon after they have been transferred to sea water and has not been linked to the freshwater environment or stock origin. Based on these observations, McVicar (1987) concluded that pancreas disease is purely a marine disease. Fish usually start to exhibit clinical pancreas disease about 6 to 12 wk after introduction to netpens, but fish that have been in pens as long as two years may be affected (McVicar 1987). Often close to 100% of a population is affected, but most fish recover and return to normal feeding and growth in a few months (Munro et al. 1984; McVicar 1987). Overall mortality due to pancreas disease, therefore, is usually low, but surviving fish may grow poorly and may be more susceptible to other diseases (McVicar and Munro 1987).

CLINICAL SIGNS AND GROSS PATHOLOGY. Affected fish are anorexic, lethargic and hang listlessly at the sides of cages near the surface. Fish with pancreas disease are usually emaciated (Fig. 40, pg. 67). Internal examination often reveals hemorrhages in the pancreatic tissue and fat between the pyloric caeca (Fig. 40), or the tissue between the pyloric caeca may be severely atrophied.

MICROSCOPIC CHANGES. Fish with pancreas disease exhibit acute and generalized necrosis of the pancreatic acinar cells. The pancreatic tissue is markedly reduced and may be replaced by a marked increase in cellularity, suggestive of inflammation, stromal condensation, and fibrosis (Ferguson et al. 1986a). Some fish may also exhibit degenerative changes in the heart, with coagulative necrosis of the ventricular myocardium (Ferguson et al. 1986a). In the post-acute (recovery) phase, fish may exhibit islands of regenerating acinar tissue amongst fibrotic tissue (Munro et al. 1984).

DIAGNOSIS. Hemorrhages in tissues associated with the pyloric caeca in emaciated Atlantic salmon smolts, along with the absence of a readily detectable infectious agent (e.g., IHN or IPN viruses, *A. salmonicida* or *Vibrio* spp.), is indicative of pancreas disease. Confirmation of the disease is based on observation of the histological changes described above.

CONTROL AND TREATMENT. No treatment is known for pancreas disease. Reports from Scotland indicate that reducing stressors (e.g., transport and handling) during the acute phase of the disease may enhance recovery. In addition, some farmers have reported that keeping fish on a smaller pellet size lessens anorexia and reduces the overall mortality associated with the disease.

Water Belly (Bloat)

Distention of the abdomen due to massive fluid accumulation in the stomach has been observed in pen-reared Atlantic salmon, chinook and coho throughout the Pacific Northwest. The syndrome has also been observed in pen-reared rainbow trout in Europe (Staurnes et al. 1990). Apparently the fluid in the stomach is primarily sea water. The cause of water belly is unknown, but some have suggested that it is exacerbated by rapid changes in pellet size or overfeeding. Staurnes et al. (1990) proposed that water belly may be related to disturbances in fat and carbohydrate metabolism.

CLINICAL SIGNS AND GROSS PATHOLOGY. Although water belly has been associated with morbidity and mortality, affected fish often appear otherwise normal and can survive for weeks with the condition. Fish with water belly exhibit severe distention of the abdominal wall. Dissection reveals a massively enlarged stomach with a very thin wall. The stomach is filled with watery, clear fluid. Atrophy of the liver has also been observed in affected fish (Hicks 1989).

MICROSCOPY. Fish do not exhibit any significant histological changes, other than thinning of the stomach wall.

DIAGNOSIS. The disease is diagnosed by observation of massive accumulation of watery, clear fluid in the stomach.

CONTROL AND TREATMENT. Some farmers have reported that decreasing the feeding rate may alleviate the problem (Hicks 1989).

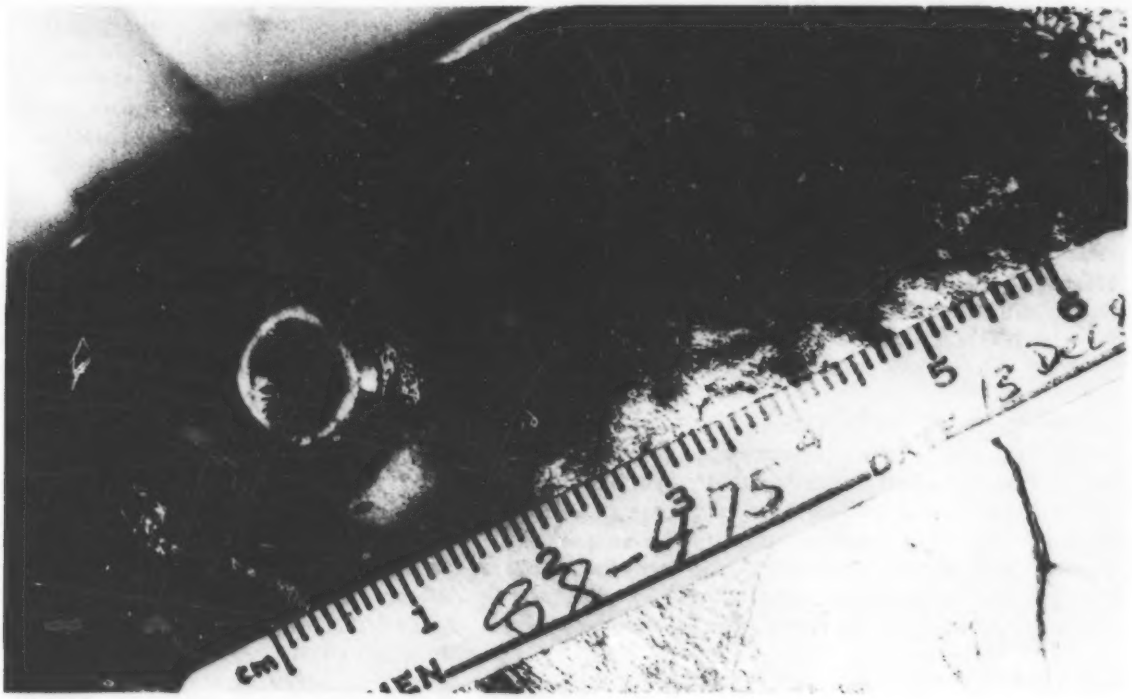


Figure 40A

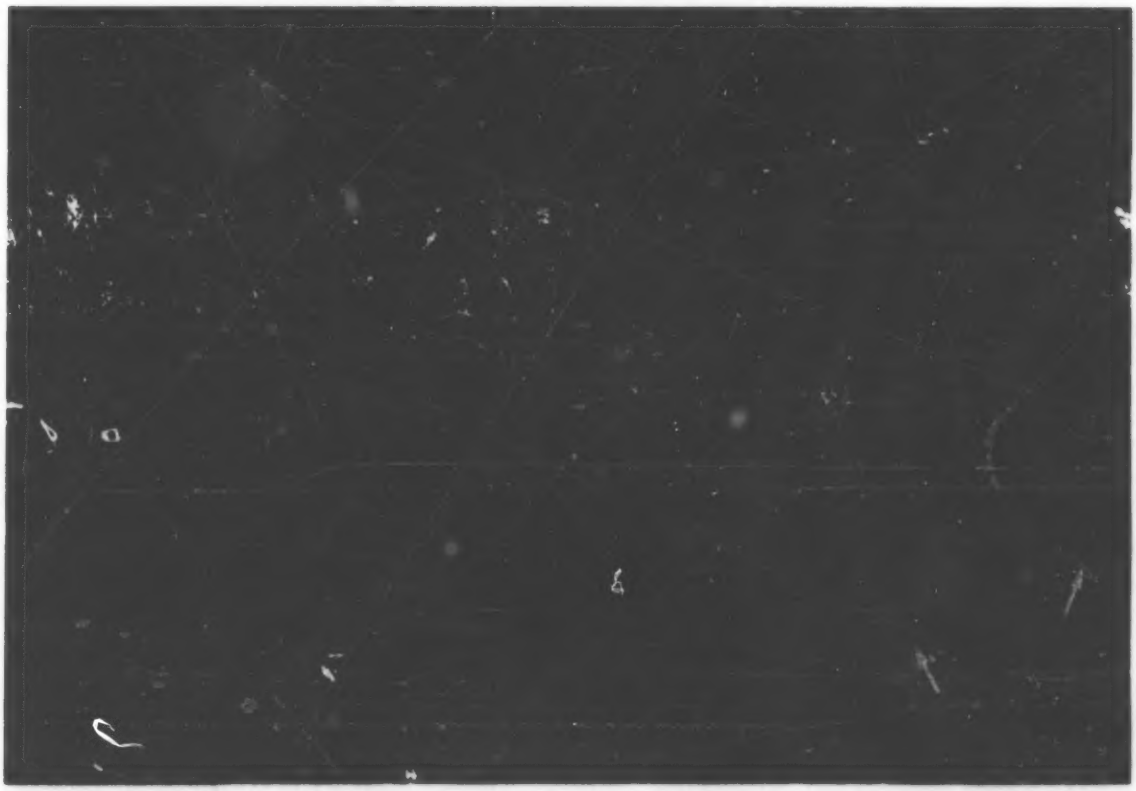


Figure 40B

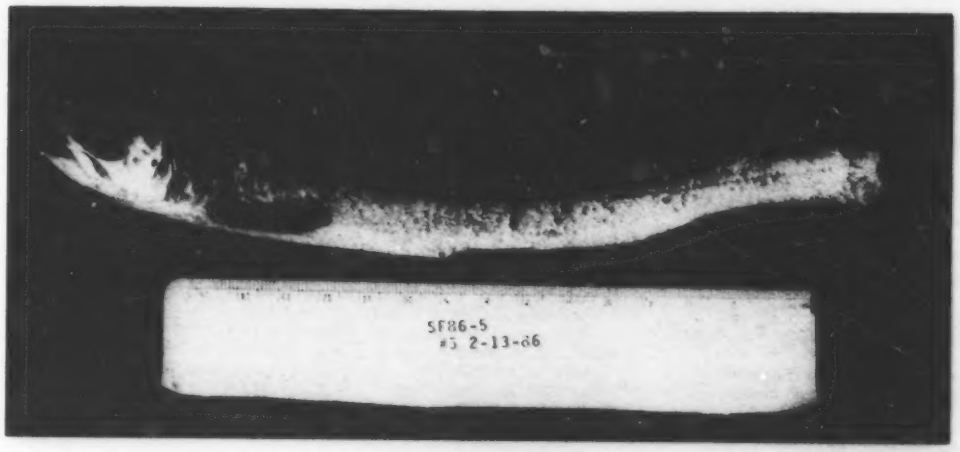


Figure 40C

Figure 40D

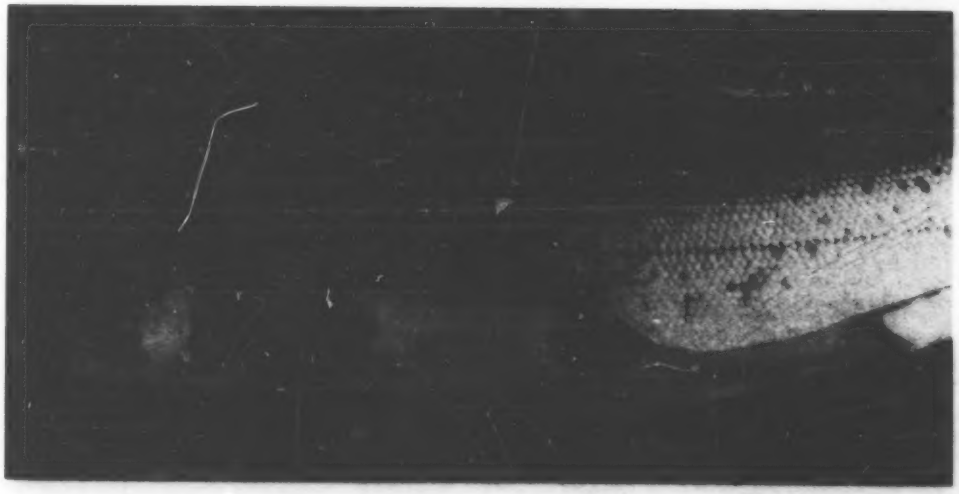


Figure 40E

Figure 40. Idiopathic diseases of pen-reared salmon. A, B. Plasmacytoid leukemia in chinook. A. Exophthalmus due to retrobulbar infiltration of plasmablasts. B. Enlargement of the spleen (S), kidney (K) and lower intestine (L), and bloody ascites (arrow) in an affected chinook. C, D. Pancreas disease in Atlantic salmon smolts. C. Emaciated smolts. D. Petechiae in the pancreas and fat associated with the pyloric caeca. E. Atlantic salmon with netpen liver disease. Note atrophied, yellow liver.

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APPENDIX I - GLOSSARY

Amphophilic. Staining readily with both acid or basic dyes. Amphophilic material usually is purple in hematoxylin and eosin-stained tissue sections.

Anemia. Reduction below normal levels of red blood cell mass in the circulatory system. Anemia is caused by hemorrhage (hemorrhagic anemia), increased red blood cell destruction (hemolytic anemia) and suppression of hemopoiesis (aplastic anemia). Nutritional deficiencies (e.g. iron, folic acid or vitamin B_{12} deficiencies) often cause aplastic anemia.

Ascites. Accumulation of serous fluid in the peritoneal cavity. A form of edema.

Ataxia. A loss of muscular coordination with impaired balance.

Atrophy. Reduction in mass of a tissue or organ, due to a reduction in cell number or cell size.

Autolysis. The initial degenerative changes in dead cells as a result of the action of endogenous enzymes. Postmortem autolysis occurs after the animal has died.

Basophilic. Having an affinity to basophilic dyes. In hematoxylin and eosin-stained tissue sections basophilic material stains blue.

Caseation. Caseous necrosis. Formation of necrotic tissue which, due to its focal nature, is soft, pasty or cheese-like.

Cataract. A loss of transparency in the lens of the eye.

Cataractous Changes. Denoting pathological changes associated with the formation of cataracts.

Ceroid. Wax-like, golden or yellow-brown intracellular material. A type of lipofuscin. Ceroid is usually formed from remaining indigestible breakdown products in phagolysosomes.

Coelozoic. Living within the lumina (tissue spaces) of organs, such as the lumina of renal tubules or the gall bladder.

Coracidia. Plural for coracidium, a larva with a ciliated epithelium that hatches from the egg of certain tapeworms.

Definitive host. Host in which a parasite achieves sexual maturity.

Digitiform. Finger-like.

Ecchymotic. Denoting hemorrhages larger than petechiae, often up to 2-3 cm.

Edema. Accumulation of abnormal amounts of fluid in the intercellular tissue spaces. Edema can be caused by decreased plasma osmotic pressure, increased blood pressure, lymphatic obstruction, or increased capillary permeability.

Eosinophilic. Having an affinity to acidic dyes. In hematoxylin and eosin-stained tissue sections eosinophilic material stains orange or red.

Epizootic. Denoting a disease attacking many animals in a population simultaneously.

Erythema. Redness of the skin.

Etiology. Causation; the study of the cause of disease.

Exophthalmus. Protrusion of the eye; "pop-eye".

Fibroplasia. Abnormal, non-neoplastic increase in fibrous tissue.

Friable. Having a gritty texture. Falls apart when handled and is easily reduced to powder.

Glomerulus. A plexus of capillaries at the beginning of each uriniferous tubule in the kidney.

Golgi body. A complex of membranes in the cytoplasm of a cell, which is distributed in a parallel orientation adjacent to the nucleus. This organelle probably functions as a site of production and packaging of cellular secretions.

Granuloma. Focal accumulation of inflammatory cells comprised primarily of macrophages. Formation of granulomas is associated with chronic inflammation.

Hemoblast. Immature blood cell.

Hemopoiesis. The process of development and formation of blood cells.

Hemorrhage. The escape of blood from the cardiovascular system.

Hemosiderin. A storage form of iron. Hemosiderin often accumulates in the liver, spleen, or kidney interstitium, and is associated with the release of iron from lysed red blood cells.

Hematoma. A localized mass of extravascular blood that is confined within an organ, tissue or space. The blood is usually clotted.

Hepatic megalocytosis. Megalocytic hepatosis. The formation of hypertrophied hepatocytes (liver cells) with greatly enlarged, often dark-staining, nuclei. This condition is apparently due to a failure of cell division in mitosis, resulting in polyploid cells. Hepatic megalocytosis is an indication of cellular toxicity, and can be induced by exposure to anthropogenic (man-made) contaminants or natural toxins.

Hepatotoxicant. A toxicant that effects the liver.

Helminth. Denoting a worm. Helminth parasites include digenean trematodes, cestodes, monogeneans, nematodes, and acanthocephalans.

Histozoic. Living within tissues outside of the cell.

Hydropic degeneration. A degenerative process at the cellular level that results in the formation of many vacuoles of variable size in the cytoplasm. Commonly the vacuoles represent distended organelles, especially the endoplasmic reticulum.

Hyperemic. Showing increased quantity of blood causing tissue to be more red than normal.

Hyperplasia. An increase in the number of normal cells. Hyperplasia will not progress, and usually resolves itself, in the absence of the causative stimulus (this is an important distinction from neoplasia).

Hypertrophy. Enlargement of individual cells or an organ.

Idiopathic. Denoting a disease of unknown cause.

Intermediate host. Host in which a parasite develops to some

extent but not to sexual maturity.

Juxtanuclear hof. A clear staining area adjacent to the nucleus representing the Golgi body.

Lamina propria. The stratum of connective tissue and associated cells underlying the epithelium of various organs, including the gut.

Leucopenia. Any situation when the total number of circulating white blood cells are less than normal.

Leukemia. Progressive proliferation of abnormal blood cells found in the hemopoietic tissues, other organs, and usually resulting in increased numbers of these cells in the blood. Leukemias are considered to be neoplasms of the blood and blood forming tissues.

Lumina. Plural for lumen, the space in the interior of a tubular structure such as a kidney tubule or artery.

Lunules. Disc-like cups on the ventral surface of the anterior margin of the body in *Caligus* spp. (parasitic copepods).

Lymphocyte. The white blood cell responsible for the immune system. Approximately 95% of salmonid fish white blood cells are lymphocytes. The cell contains a large nucleus and a narrow rim of basophilic cytoplasm.

Lymphoblast. Immature lymphocyte.

Melanomacrophage. A macrophage containing melanin. As with normal macrophages, increase of these cells is often associated with chronic inflammation.

Melanin. Black or brown pigment thought to play a role in the detoxification of free radicals and cations. Melanin is also an important pigment in skin color.

Metacercaria. Stage between the cercaria and adult in the life cycle of most digenetic trematodes. Usually encysted and quiescent. This stage is absent in blood flukes.

Metacestode. Juvenile developmental stage of a cestode. In fish, this stage is usually equivalent to the pleurocercoid.

Macrophage. A large, mononuclear phagocytic white blood cell. Macrophages engulf foreign material, including pathogens, and are associated with chronic inflammation. Fixed macrophages (=reticuloendothelial system) are abundant in the kidney interstitium, atrium of the heart, and spleen.

Macroscopic. Visible with the naked eye.

Megalocytosis. See hepatic megalocytosis.

Merogony. Developmental phase of certain protozoans (e.g., coccidians, microsporidians) involving asexual, vegetative proliferation.

Miracidium. The first larval stage of digenean trematodes. Miracidia infect the first intermediate hosts of digenean trematodes, which are usually molluscs. Miracidia are small, ciliated, and swim actively.

Multinucleate giant cell. A syncytia of macrophages. This cell is often found in granulomas of higher animals and occasionally in fish.

Necrosis. Cell death within a living organism.

Neoplasia. The pathologic process resulting in the growth of a neoplasm.

Neoplasm. An abnormal mass of tissue, the growth of which exceeds and is uncoordinated with that of normal tissues, and persists after the causative stimulus is removed.

Nephritis. Inflammation of the kidney.

Neutrophil. A white blood cell similar in morphology to neutrophils of higher animals. The cytoplasm is filled with fine granules and the nucleus is lobate and segmented. Although this cell is important in acute inflammation in mammals, the cell is relatively rare in fish and its function in fish is not entirely clear.

Oncogenic. Denoting an agent that causes neoplasia.

Panophthalmitis. Inflammation throughout the eyeball.

Pathognomonic. Characteristic or indicative of a disease.

Petechiae. Minute, focal hemorrhages up to 1 to 2 mm.

Plasma cell. Mature B lymphocyte that has a well organize rough endoplasmic reticulum and produces a relatively large amount of antibody.

Plasmablast. Immature plasma cell.

Plerocercoid. Cestode larva that develops from a procercoid. This stage is often found in the tissues of fishes.

Procercoid. Cestode larva that usually infects the first intermediate hosts. In cestodes of aquatic animals, this host is usually a crustacean.

Pyriform. Pear-shaped.

Retrobulbar. Behind the eyeball.

Septicemia. Systemic infection of the cardiovascular system.

Serosanguinous. Denoting a discharge or exudate containing serum and blood (red-tinged fluid).

Sporogeny. A developmental phase in certain protozoans (e.g., microsporidians) that results in the formation of spores.

Stomatitis. Inflammation of the mouth.

Thrombi. Plural for thrombus, a solid mass formed within a blood vessel.

Vasculitis. Inflammation of blood vessels.

Xenoma. A host cell packed with microsporidian parasites and that is greatly hypertrophied. The host cell nucleus is also greatly hypertrophied, which may be branched or fragmented.

APPENDIX II - SCIENTIFIC NAMES OF FISHES

Amago salmon - *Oncorhynchus rhodurus*
Atlantic salmon - *Salmo salar*
Atlantic herring - *Clupea harengus*
Atlantic cod - *Gadus morhua*
Big skate - *Raja binoculata*
Bluegill sunfish - *Lepomis macrochirus*
Brook trout - *Salvelinus fontinalis*
Brown trout - *Salmo trutta*
Chinook - *Oncorhynchus tshawytscha*
Chum salmon - *Oncorhynchus keta*
Coho - *Oncorhynchus kisutch*
Copper rockfish - *Sebastes caurinus*
Cutthroat trout - *Oncorhgnchus clarki*
Greenling - *Hexagrammos sp.*
Haddock - *Melanogrammus aeglefinus*
Japanese flounder - *Paralichthys olivaceus*
Japanese yellowtail - *Seriola quinqueradiata*
Kokanee salmon - *Oncorhynchus nerka*
Masou salmon - *Oncorhynchus masou*
North Sea whiting - *Merlangius merlangus*
Pacific cod - *Gadus macrocephalus*
Pacific herring - *Clupea pallasi*
Pacific hake - *Merluccius productus*
Pink salmon - *Oncorhynchus gorbuscha*
Plaice - *Pleuronectes platessa*
Rainbow trout - *Oncorhynchus mykiss*
Siamese fighting fish - *Betta splendens*
Sockeye salmon - *Oncorhynchus nerka*
Speckled sanddab - *Citharichthys stigmaeus*
Spiny dogfish - *Squalus acanthias*
Spotted ratfish - *Hydrolagus colliei*
Steelhead trout - *Oncorhynchus mykiss*
Threespine stickleback - *Gasterosteus aculeatus*
Walleye pollock - *Theragra chalcogramma*

APPENDIX III - PRESERVATIVES AND CULTURE MEDIA

Lugol's Preservative

Dissolve 100 g potassium iodide in 1 L distilled water. Then dissolve 50 g crystalline iodine into the solution. Finally, add 100 ml glacial acetic acid. Any precipitate is removed by filtration or decanting. Lugol's is added to the sample until the sample has a weak tea color (about 1 drop/ml).

Davidson's solution

95% ethanol	300 ml
formalin	200 ml
glacial acetic acid	100 ml
distilled water	300 ml

50% Seawater-Cytophaga Medium

tryptone	0.5 g
yeast extract	0.5 g
sodium acetate	0.2 g
beef extract	0.2 g
agar	11.0 g

add above to:

filtered sea water	500 ml
distilled water	500 ml

autoclave and pore into sterile petri dishes.

LEISHMAN'S GIEMSA

This stain is useful for examining protozoa, bacteria and cell morphology in tissue imprints.

Leishman's. Add 1 g Leishman's stain to 500 ml absolute methanol and filter.

Giemsa Stock Solution. Add 1 g powder to 66 ml to glycerol and heat at 60°C for one hour. Then add 66 ml of absolute methanol after the solution has cooled, and filter. Store at 4°C.

Phosphate Buffer Solutions.

Solution A - 31.20 g $NaH_2PO_4 \cdot 2\ H_20$ in 1 L distilled water

Solution B - 53.65 g $Na_2HPO_4 \cdot 7\ H_20$ in 1 L distilled water

Giemsa Working Solution.

To prepare phospate buffer, mix 73.5 ml solution A with 26.5 ml solution B. Then add 100 ml distilled water.

Add 3.5 ml stock solution to 50 ml phosphate buffer (pH 6.4). Make fresh for each use.

Staining Procedure:

1. Prepare smear or imprint and allow to dry for about 1/2 hour.
2. Fix in methanol for 2-5 min.
3. Stain in Leishman's for 2-3 min.
4. Stain in Giemsa for 10-12 min.
5. Rinse in distilled water for about 1 min.
6. Air dry.